GENETIC STRUCTURE, DIVERSITY AND EVOLUTIONARY HISTORY OF THE IVORY GULL (PAGOPHILA EBURNEA) AND ROSS'S GULL (RHODOSTETHIA ROSEA):

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GENETIC STRUCTURE, DIVERSITY AND EVOLUTIONARY HISTORY OF THE IVORY GULL (*PAGOPHILA EBURNEA*) AND ROSS'S GULL (*RHODOSTETHIA ROSEA*):

by

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Abstract

The Ivory Gull (*Pagophila eburnea*) is classified as "Endangered" under the Species at Risk Act in Canada due to an 80% decline in population survey counts at known breeding sites between the early 1980s and 2003-2005. This study aimed to fill critical information gaps with regard to the Ivory Gull's global population structure, with a genomic approach of several mitochondrial gene sequences. Ivory Gulls have a low level of genetic diversity, similar to other endangered and arctic species. Most of the genetic variance is within populations, such that the Canadian, Greenland and Norwegian breeding populations are genetically indistinguishable and the source of the Labrador Sea wintering birds is unidentifiable. The Alaskan non-breeding population was weakly differentiated from the breeding colonies analyzed and the Labrador Sea wintering population.

Ross's Gull (*Rhodostethia rosea*) is classified as Threatened in Canada due to the extremely small numbers of breeding birds. A small number of museum specimens were used to analyze the control region sequence. The genetic diversity of the Canadian birds was much lower than the Alaskan individuals and the two populations were weakly differentiated although the source of this was unclear.

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Chapter 1

General Introduction

1.1 Rationale

For most declining species, ignorance of basic elements of their biology impedes conservation efforts (Ryder 2005). This is true of Ivory Gulls (*Pagophila eburnea*) and Ross's Gulls (*Rhodostethia rosea*). These birds are both of conservation concern in Canada but they are two of the most poorly studied seabird species in the world (Alvo *et al.* 1996; Gilchrist and Mallory 2005). Use of traditional field methods to obtain the required information would be tremendously difficult given their use of extremely remote Arctic breeding areas. Therefore, Ivory Gulls and Ross's Gulls were excellent models for a conservation genetics approach.

1.2 Conservation Genetics

Conservation genetics can help to provide essential information about a species' population structure and evolutionary history. For example, it is important to determine the amount of gene flow among colonies or isolated populations of a threatened species. Gene flow is defined as the transfer of genetic material between populations that results from movements of individuals (migration) or their gametes (Avise 2004). The exchange of only one to four females per generation is thought to prevent differentiation of mtDNA by drift alone (Crow and Aoki 1982). In birds, flight and the resultant high potential for dispersal may explain the absence of significant genetic differentiation frequently

observed among local breeding populations (Avise *et al.* 2000). However, many avian species show strong tendencies toward nest site philopatry, and constitute recognized subspecies with distinct geographic variation (Newton 2003). Understanding of the genetic evolutionary history would permit informed decisions about whether a species could benefit from the transplant of individuals among colonies or breeding localities to prevent inbreeding and loss of genetic diversity (Frankham, Ballou and Briscoe 2004).

One of the goals of conservation genetics is to fully understand the relationship between genetic diversity and population viability. Genetic diversity is one of three forms of biodiversity recognized by the World Conservation Union (IUCN) as deserving of conservation (Reed and Frankham 2003). The need to conserve genetic diversity within populations is based on two arguments: the necessity of genetic diversity for evolution to occur, and the expected relationship between heterozygosity and population fitness (Reed and Frankham 2003). Both of these arguments have been shown to be correct as genetic diversity is both a key parameter of a populations' likelihood of recovery (Frankham, Ballou and Briscoe 2004) and is also strongly correlated with fitness (Reed and Frankham 2003). High levels of genetic diversity are seen as healthy, allowing the population to respond to threats such as disease, parasites, predators and environmental change (Amos and Harwood 1998) whereas decreased genetic diversity has been shown to adversely affect adult longevity (Saccheri *et al.* 1998) and to increase the risk of extinction (Frankham 1998), especially during environmental stress (Frankham and Ralls 1998; Reed, Briscoe and Frankham 2002). Low genetic diversity has also been shown to result in increased egg infertility (Jamieson and Ryan 2000) and hatching failure (Bensch *et al.* 1994). However, low genetic diversity does not necessarily doom a

population to extinction as there have been cases of bird species, such as the Chatham Black Robin (*Petroica traversi*), that were bottlenecked to a single breeding pair and managed to survive (Ardern and Lambert 1997). The apparently rapidly declining Canadian population of Ivory Gulls and extremely low Canadian population of Ross's Gulls make them ideal study species for conservation-oriented research into genetic diversity.

1.2.1 Arctic Conservation Genetics

Genetic diversity is often lower for species living at higher latitudes (Martin and McKay 2004), likely as a consequence of long-term climatic oscillations that result in species repeatedly retreating from and then re-colonizing their ranges (Dynesius and Jansson 2000). The small population size associated with isolation in refugia during ice ages and subsequent rapid re-colonization also causes reduced genetic variability (Hewitt 1996). For instance, Atlantic Common Murres (*Uria aalge*), which have a northerly breeding range, showed a star-like haplotype phylogeny and little sequence divergence, suggesting a recent population expansion (Moum and Arnason 2001).

Analysis of genetic diversity can supply information about the history of a species. For example, the Razorbill (*Alca torda*) has 97% of its global population breeding outside North America (Nettleship and Evans 1985). When Moum and Arnason (2001) sequenced its control region, they found that nucleotide diversity was actually highest in the two North American colonies, which suggests that the current Razorbill population originated from a southwest Atlantic refugial population and through sequential founder events colonized the North and East Atlantic. In contrast, the Lesser

White-fronted Goose (*Anser erythropus*) has a large (25,000) breeding population in Russia as well as a very small (30-50 pairs) breeding population in Fennoscandia, which had only half the haplotype and nucleotide diversity of the main Russian population (Ruokonen *et al.* 2004). These cases illustrate that population size and genetic diversity is not always linked.

In addition, northern taxa are often subject to natural selection for high dispersal capacity, which likely leads to homogenizing gene flow over large areas (Liebers and Helbig 2002), such that species at high latitudes theoretically should show less phylogeographical population structure than closely related species further south (Dynesius and Jansson 2000). Empirically however, there is considerable variation in the amount of phylogeographic structure among Arctic species as a result of different life-history characteristics and the level of fragmentation into refugia (Avise and Walker 1998). For example, Steller's Eiders (*Polysticta stelleri*) breed mainly in Russia but a small genetically differentiated population of conservation concern breeds in Alaska (Pearce *et al.* 2005). In contrast, King Eiders (*Somateria spectabilis*) showed little evidence for genetic differentiation despite having two distinctly distributed populations with separate wintering areas (Pearce *et al.* 2004). Geographic barriers to gene flow also exist even in apparently mobile avian species. For example, among 13 bird species, including the Mew Gull (*Larus canus*), analyzed using mitochondrial DNA, 11 exhibited evidence of genetic differentiation between each side of Beringia (Zink *et al.* 1995).

1.2.2 Endangered Species Conservation Genetics

It was recently shown that small populations of threatened species frequently have lower genetic variation than populations of related species that are not threatened (Spielman, Brook and Frankham 2004) which makes determination of the phylogeography of threatened species more difficult. For instance, the endangered Crested Ibis (*Nipponia nippon*) only had two haplotypes in the control region domains II and III (n=36) (Zhang, Fang and Xi 2004). The Japanese regional population of the Oriental White Stork (*Ciconia boyciana*) was also determined to be genetically homogeneous at a 1210bp control region sequence before its extirpation (Murata *et al.* 2004). The decline of populations often results in loss of rare alleles, which ultimately leads to decreased heterozygosity that can theoretically affect the ability of the species to persist and adapt in the face of environmental change (Frankham and Ralls 1998; Reed, Briscoe and Frankham 2002). The aftermath of severe population reductions may last for many thousands of generations (Briskie and Mackintosh 2004). Theoretically, a population that has expanded from a small population (bottleneck) will show evidence of a low historical effective population size (N_e) with low haplotype and nucleotide diversities (Glenn, Stephan and Braun 1999), a star-like phylogeny of haplotypes with very low levels of population subdivision, and a unimodal distribution of pairwise differences among haplotypes (Mila *et al.* 2000). On the other hand, several authors have questioned the evidence for the deleterious effects of loss of genetic diversity, pointing to the existence of viable populations of numerous species in the absence of genetic diversity due to being bottlenecked to only a few individuals (e.g. Black Robin (Arden and Lambert 1997); Whooping Crane *Grus americana* (Glenn, Stephan and Braun 1999) and Crested Ibis (Zhang, Fang and Xi 2004)).

Genetic drift is defined as the fluctuations in allele frequency within and among populations that occurs by chance because of sampling error (Connor and Hartl 2004). The effect is particularly noticeable in small populations or as a result of founding events (Connor and Hartl 2004). The effect of genetic drift is demonstrated by the Whooping Crane, which declined from six haplotypes in the pre-bottleneck sample to only one in the modern population, as indicated by 314bp of control region data from museum specimens (Glenn, Stephan and Braun 1999). The one haplotype that persisted was at a low frequency in the pre-bottleneck population, a classic consequence of genetic drift (Glenn, Stephan and Braun 1999).

1.2.3 Gull Conservation Genetics

Compared to other seabird studied, in which the among-population genetic variance component was generally lower, gulls sometimes exhibit strong phylogeographic structure, despite their high colonization potential (Liebers, Helbig and De Kniff 2001). The Red-legged Kittiwake (*Rissa brevirostris*), a gull endemic to the Bering Sea, had a statistically significant population genetic structure in which birds from Bering Island were genetically differentiated from other colonies analyzed, likely as a result of strong nesting site fidelity (Patirana, Hatch and Friesen 2002). However, the overall level of differentiation was low, so that Red-legged Kittiwakes can still be considered a single management unit (Patirana, Hatch and Friesen 2002). Analysis of genetic markers in Black-legged Kittiwakes (*Rissa tridactyla*) showed considerably more genetic structure as the Pacific and Atlantic populations were significantly different from each other (Patriana 2000). Several differentiated colonies were found in the Atlantic whereas the Pacific

colonies were not differentiated from each other, and most of the variance was distributed within populations (Patirana 2000). No significant microsatellite variation was found between the two largest colonies of Audouin's Gulls (*Larus audouinii*), and they appear to be a panmictic population despite being relatively philopatric and having different body sizes (Genovart, Oro and Bonhomme 2003). A panmictic population is one where all individuals are potential partners as a result of being free to move within their habitat without any sort of geographical or behavioral restrictions. Auduoin's Gull is restricted to the Mediterranean Sea and may always have had a small total population size compared to the Black-legged Kittiwake.

It has been argued that species at high latitudes should show less phylogeographical population structure and thus be less likely to speciate than closely related species further south (Dynesius and Jansson 2000). This has be shown to be the case with Lesser Black-backed Gull complex (Liebers, Helbig and De Kniff 2001; Liebers and Helbig 2002), which contains the southerly distributed Yellow-legged Gull *Larus cachinnans* (6 subspecies), the northerly distributed Lesser Black-backed Gull *Larus fuscus* (5 subspecies) and the Herring Gull *Larus argentatus* (3 subspecies). Lesser Black-backed Gulls were characterized by a star-like haplotype phylogeny centered on two highly dominating haplotypes, while many rare haplotypes differed by only single substitutions (Liebers and Helbig 2002). In contrast, Yellow-legged Gulls showed a complex haplotype network with multiple, divergent clusters, corresponding to long periods of multiregional differentiation (Liebers, Helbig and De Kniff 2001).

Gull species have varying degrees of genetic diversity. Some gull species of conservation concern have been shown to have low levels of genetic variation, such as

Audouin's Gulls, which had no variation in the 16 individuals sequenced for 500bp from domains II and III of the control region (Genovart, Oro and Bonhomme 2003). However, Red-legged Kittiwakes, also of conservation concern, had high levels of genetic diversity in domain I of the control region (Patirana, Hatch and Friesen 2002). Several taxa, such as the Mongolian Gull (*Larus mongolicus*) and Armenian Gull (*Larus armenicus*) show little mitochondrial genetic diversity, likely as a result of recent population expansion from a bottleneck (Liebers, Helbig and De Kniff 2001). Current population size is not always a predictor of genetic diversity as Herring Gulls, one of the most abundant gulls in North America, had low cytochrome b sequence divergence in the Great Lakes region (Chen *et al.* 2001). Black-legged Kittiwakes, which are not of conservation concern, more predictably had high levels of genetic diversity, as indicated by 155 haplotypes defined by 115 variable positions, mostly in domain I, among 404 samples using 773 bp of control region sequence (Patirana 2000).

1.3 Mitochondrial DNA

1.3.1 Properties

Vertebrate mitochondrial DNA is the most widely used genetic marker for phylogeography and has been used in more than 80% of published studies (Avise 1998). There are several advantages to the use of mtDNA for conservation genetics. It is maternally inherited (Lansman, Avise and Huettel 1983) and there is no direct evidence that it can recombine with other mitochondrial genomes (Hayashi, Tagoshira and Yoshida 1985). However, the maternal inheritance of mitochondria means that it only provides information on female dispersal and matrilineal phylogeography, unlike nuclear DNA,

which can provide data on both male and female genetic transmission (Avise 1995). Mitochondrial DNA has been shown to evolve more quickly than most nuclear DNA (Brown, George and Wilson 1979). Also, because the effective population size of mtDNA is 1/4 that of nuclear DNA, mtDNA is more sensitive to population bottlenecks and gene flow restrictions than is nuclear DNA (Wilson *et al.* 1985). As a result, the amount of variation of mtDNA can be presumed to reflect the amount of variation in nuclear DNA, in the absence of selection (Wilson *et al.* 1985). Mitochondrial DNA is only a single genetic locus, however, and reliance on a single locus weakens the ability to detect significant spatial or temporal patterns. For this reason sequencing of several loci provides the most insight into historical processes (Palumbi and Baker 1994). Mitochondrial DNA is also present in much higher copy number than nuclear DNA (Ballard and Whitlock 2004), making it easier to amplify from suboptimal DNA extracts (Cooper 1994), such as museum specimens.

Museum specimens are valuable sources of genetic material for rare birds that are difficult to collect from the field (Payne and Sorenson 2002). Shorter fragments of DNA are more likely to survive in museum specimens and increase the chance of successful amplification (Glenn, Stephan and Braun 1999). Conservation genetic studies often utilize museum specimens to monitor temporal trends in genetic diversity (Sefc, Payne and Sorenson 2007). However, museum specimen mtDNA can contain artifactual base changes (at approximately 1×10^{-4} per base pair) which may bias towards a higher haplotype diversity in historical samples as compared with current populations (Sefc, Payne and Sorenson 2007). The use of museum specimens has provided essential data on the population structure of birds, for example studies on the Loggerhead Shrike *Lanius*

ludovicianus (Vallianatos, Lougheed and Boag 2002); Oriental White Stork *Ciconia boyciana* (Murata *et al.* 2004); Heath Hen *Tympanuchus cupido* (Ross *et al.* 2006); and Red Grouse *Lagopus lagopus scoticus* (Freeland *et al.* 2006).

1.3.2 Mitochondrial Gene Regions

All vertebrate mitochondrial genomes contain 22 tRNA-coding regions, 13 protein-coding regions, 2 rRNA-coding regions and the Control Region (Clayton 1992). There is considerable variation in the mutation rates within and among the different gene regions (Mindell and Thacker 1996). To improve the power of analysis, separate sites throughout the genome need to be examined because one continuous region will not accurately represent the entire genome (Cummings, Otto and Wakeley 1995).

The Control Region or D-Loop Region is often considered to be the most variable region of mtDNA, in terms of nucleotide substitutions, short insertions/deletions (indels) and dynamics of variable-number tandem repeats (Randi and Lucchini 1998). The Control Region is a non-coding region that regulates replication of the heavy strand (which has a higher [G + C] content than the light strand) and transcription of the mtDNA genome (Clayton 1992). This non-coding region is usually divided into three subregions: Domain II is a central, more conserved domain that is flanked by Domains I and III, which show substantial size and sequence variation (Marshall and Baker 1997). In gulls (Laridae), the higher rate of base substitution is particularly true of Control Region Domain I sequences which evolve much more quickly than Domains II and III (Crochet and Desmarais 2000). However, the Control Region is not always the most variable region of the mitochondrial genome. When the Control Region and Cytochrome *b*

divergence were compared in many avian species, Ruokonen and Kvist (2002) found that the variability ratios varied from 0.13 to 21.65, suggesting that there are differences in the rate of divergences among avian lineages.

12S rRNA is the smaller of two mitochondrial ribosomal DNAs and together with the 16S subunit complexes with proteins to form a ribosome (Houde *et al.* 1997). Because it is a non-protein-coding molecule, more variation at the nucleotide level is possible as compared to a protein-coding gene (van der Kuryl *et al.* 1995). However, some selective pressure does still act upon the gene to maintain the correct secondary structure necessary for rRNA function (van der Kuryl *et al.* 1995). Therefore, 12S rRNA includes both evolutionary labile and conserved regions and can permit assessment of recent and ancient divergences (Houde *et al.* 1997). Control Region and 12S rRNA sequence data were used to determine population structure of Andean Condors (*Vultur gryphus*) and it was found that both regions contributed important SNPs (single nucleotide polymorphisms) to the analysis (Hendrickson *et al.* 2003). ND4 has been found to provide a higher proportion of variable and informative sites than Cytochrome *b*, which is often used in genetic analysis (Feldman and Omland 2005).

1.4 Ivory Gull Natural History

The Ivory Gull is the only all-white gull with black legs and is smaller than other white-headed gulls (448-687g) (Haney and McDonald 1995). Juvenile (and first-winter) Ivory Gulls have black blotches on the face, wings and tail, which gives them a characteristic 'ermine' appearance. They develop the pure white adult plumage in their second year (Haney and McDonald 1995). Ivory Gulls are the sole member of their

genus, *Pagophila*. Mitochondrial DNA sequence analysis determined that their closest sister taxon is Sabine's Gull (*Xema sabini*) followed by kittiwakes (*Rissa* spp.) (Crochet, Bonhomme and Lebreton 2000; Pons, Hassanin and Crochet 2005).

1.4.1 Status

The Canadian population of Ivory Gulls was assessed as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2006 after previously being designated as Special Concern. They are also classified as Rare (Category 3) in the Red Data Book of Russia (Zubakin 1984) and Declining in Svalbard (Anker-Nilssen *et al.* 2000). The Ivory Gull has been recommended as an indicator species for the health of the arctic marine environment by scientists of the circumpolar community (Mallory and Gilchrist 2005).

1.4.2 Movement

Band recoveries indicate that Ivory Gulls are capable of moving long distances from where they were banded but the sample size was very low, and it is unknown how general this trend is (Cramp and Simmons 1983). A basic question concerns the breeding colony origin of northwest Atlantic and Bering Sea wintering birds. It has been assumed that birds wintering in the northwest Atlantic (mostly in pack ice off Labrador and Newfoundland) originate at arctic Canadian, Greenland and possibly Norwegian breeding colonies but there are little data available to support this (Haney and McDonald 1995). Origins of Ivory Gulls wintering in the Bering Sea are unknown, but a male banded at Franz Josef Land, Russia was later recovered south of the Chukchi Peninsula, which

suggests that birds from the Russian Arctic colonies may winter off the Bering Sea coasts of Russia and Alaska (Tomkovich 1990). Sightings of juveniles during fall and winter near Tuktoyaktuk, NWT (Porsild 1943), suggest that birds from the eastern Canadian high Arctic might also wander occasionally into the Beaufort, Chukchi and Bering Seas (Haney 1993).

1.4.3 Canadian Population

The Canadian breeding population size and distribution seems to have been shrinking since the late 1800s (Haney and MacDonald 1995) but in the past 10-15 years an 80% decline in counts of birds at known colony sites has been documented (Gilchrist and Mallory 2005). Sparse data from at-sea observations are also consistent with a considerable decline, as four times more Ivory Gulls were seen in 1993 than in 2002 (Chardine *et al.* 2004). Ivory Gulls formerly bred in northwestern Canada on Bathurst Island, the Polynya Islands, and on Prince Patrick Island at the edge of the Beaufort Sea (MacDonald and MacPherson 1962). Currently, there are Ivory Gull breeding colonies on Ellesmere Island, Seymour Island, Devon Island, Perley Island and Baffin Island (Haney and McDonald 1995). Canada was thought to support as much as 6-10% (2400 individuals) of the global breeding population of 14,000 pairs by Volkov and de Korte (1996), but Gilchrist and Mallory (2005) suggest the Canadian breeding population may be only 250-350 pairs. No study of Ivory Gull breeding biology has been performed in Canada since MacDonald (1976).

1.4.4 World Population

Similar declines of Ivory Gull populations are suspected in other regions but unfortunately, Russian and Norwegian fieldwork largely stopped in the mid-1990s due to monetary difficulties (Krajick 2003). Several thousand birds were estimated for Franz Josef Land, Russia in the early 1900s (Haney and McDonald 1995); however no colonies were found in a 1996 survey of known nesting sites in a major breeding region (Krajick 2003). The most recent estimates of Ivory Gulls in Severnaya Zemlya, Russia indicated that 1000-2000 birds bred there (Haney and MacDonald 1995). These data indicate that the last Russian estimate of 10,000 breeding pairs is likely unrealistic and the real population is probably much lower (Krajick 2003). Ivory Gulls have declined in Spitsbergen since the nineteenth century when colonies of 100 or more pairs were often recorded (Bateson and Plowright 1959). The last population estimates made in the 1960s found only 344 pairs (Birkenmajer 1969). More than 200 birds were banded in the summer of 2003 in Greenland but many areas of Greenland are not well explored, so the suggested stable population size of 1,000 birds could be too high or too low (Krajick 2003). There is no evidence that Ivory Gulls have ever bred in Alaska (Haney and McDonald 1995).

During late winter and early spring of 1978-1979, Ivory Gulls were estimated to number about 35,000 from aerial censuses over Davis Strait, their main wintering area (Orr and Parsons 1982). However, because this estimate was derived from sampling a relatively small area of the Davis Strait and Labrador Sea, no confidence limits on the estimate were presented, and this may be a major overestimate (Stenhouse 2003). Vuilleumier (1995) suggests that even if the generous estimates of breeding birds in Canada and Russia (several thousand each) were accurate, which is unlikely, the global

population could not exceed 10,000 breeding birds, making it one of the rarest Arctic seabirds.

1.5 Ross's Gull Natural History

Ross's Gull is a small gull with a black neck collar, wedge-shaped tail and pink underpart colouration that is displayed during the breeding season (Densley 1999). Recent phylogenetic analysis based on mtDNA has shown that Ross's Gull is a sister taxon to the Little Gull (*Larus minutus;* Pons, Hassanin and Crochet 2005).

1.5.1 Status and Research

Ross's Gull was listed as a 'Vulnerable' species in 1981 by COSEWIC and was then up-listed in 2001 to 'Threatened' because of its small population size and low productivity (Alvo *et al.* 1996).

Very little is known about Ross's Gull ecology. Ross's Gulls have an extremely low nesting concentration that is a special form of low density colonial nesting within the framework of coloniality (Zubakin and Avdanin 1983). This species' area of greatest breeding season abundance is in coastal low Arctic and taiga regions of Russia, mostly between the Khroma and Kolyma rivers in northeastern Siberia (Cramp and Simmons 1983) and Buturlin (1906) did the most in-depth work on its breeding biology. Canadian populations are peripheral and occupy atypical habitat compared to Russian populations (Alvo *et al.* 1996). Ross's Gulls appear to move colonies each year or occupy colonies intermittently, especially in the Canadian High Arctic and it is possible that Ross's Gulls

do not breed every year, perhaps due to food supply (Mallory, Gilchrist and Mallory 2006).

The highly productive polar ice that borders the Barents and Greenland Seas serves as an important feeding and moulting area for non-breeding Ross's Gulls during the summer (Meltofte *et al.* 1981). In fact, Ross's Gulls appear to be the most common bird in the central Arctic Ocean, north of 85°N (Hjort, Gudmundsson and Elander 1997). During the fall, large numbers of these gulls pass eastward by Point Barrow, Alaska to feed in the Arctic Ocean (Divoky 1976) and for a long time it was unknown if they stayed that far north during the winter. It was subsequently shown that they return westward and winter in the Bering Sea and the Sea of Okhotsk (Degtyarev, Labutin and Blohin 1987).

1.5.2 Canadian Population

Ross's Gull is the rarest breeding gull in North America, where only four breeding locations have been confirmed: in Nunavut at the Cheyne Islands (McDonald 1978); Prince Charles Island (Bechet *et al.* 2000); unnamed island in Penny Strait (Mallory, Gilchrist and Mallory 2006); and Churchill in Manitoba (Chartier and Cooke 1980). The Canadian population of Ross's Gull is thought to have always been small, despite large areas of potential habitat (Alvo *et al.* 1996). The species appears to have nested annually or almost annually from 1980 to 1994 in Churchill, Manitoba and/or in Nunavut and the known breeding population has varied from 1 to 5 pairs (Alvo *et al.* 1996). Ross's Gulls are known to many Inuit in southern Baffin Island, indicating they may be more common than previously thought (Mallory, Gilchrist and Mallory 2006).

1.5.3 World Population

Ross's Gull is still considered a high-arctic gull, despite 95% or more of its breeding population being found in the marshy wetlands of northeastern Siberia, between the Chukotka and Taymyr Peninsulas (Hjort, Gudmundsson and Elander 1997). The world population calculated in 1978 at 10,000 is thought to have been underestimated with recent censuses of Siberian breeding grounds revealing that the world population may be as many as 50,000 (Alvo *et al.* 1996). However, according to a recent survey of northern Yakutia, Russia, Ross's Gull is more widespread than had ever been assumed as their current population estimate of 100,000 birds might be low, making it much larger than any previous estimate (CAFF 2004). There are many sightings and several reports of Ross's Gulls breeding in Greenland but it is unknown whether these birds represent a breeding population or are just isolated vagrants (Kampp and Kristensen 1980).

1.6 Ivory Gull and Ross's Gull Conservation

Since Ivory Gull declines have occurred across the known Canadian breeding range, the cause of the decline has been suggested to be related to factors involved in migration or their wintering area (Gilchrist and Mallory 2005). Declines may also be due to factors that birds in many breeding areas have in common, for example hunting, climate change, contamination, or disturbance.

1.6.1 Hunting

Hunting is likely an important contributing factor to the Ivory Gulls' decline. Ivory Gulls are now legally protected throughout Canada but have been traditionally shot for

food and recreation in Nunavut during the summer and off the northeast coast of Newfoundland during winter (Stenhouse, Robertson and Gilchrist 2004). Several of the band returns of the n=1526 Ivory Gulls banded in Arctic Canada during the 1970s and 1980s came from birds shot in Canada (n=5) or northwest Greenland (n=17) during the spring and fall migrations (Stenhouse, Robertson and Gilchrist 2004). Interestingly, no birds were recovered from Greenland in August, which is the peak breeding month and recoveries of first- and second-year birds were also not common (Stenhouse, Robertson and Gilchrist 2004). This evidence suggests that Ivory Gulls are vulnerable during their pre- and post-breeding movements and younger birds may not participate as much in these movements (Stenhouse, Robertson and Gilchrist 2004). Birds that breed in the northern part of Canada and in northern Greenland seem to be the most vulnerable to hunting mortality as their migration route takes them past areas of strong hunting pressure (Stenhouse, Robertson and Gilchrist 2004). Intense and unregulated hunting also occurs on the eastern coast of Russia, where Ivory Gulls often occur during the winter (Greg Robertson, personal communication). Recovery rates for Ivory Gulls were relatively high (0.03 ± 0.009) and similar to other harvested seabirds such as the Thick-billed Murre (*Uria lomvia*) in west Greenland (Stenhouse, Robertson and Gilchrist 2004), despite protection against hunting since 1989. The reduction in life expectancy as a result of hunting means fewer reproductive opportunities and thus lower reproductive success which could have significant effects to a species like the Ivory Gull, which may be forced to abandon breeding in some years due to the harsh climate of the High Arctic (Stenhouse, Robertson and Gilchrist 2004).

1.6.2 Climate Change

Global warming would seem to be a prime suspect as Ivory Gulls feed in association with sea-ice year-round and so are likely dependent on it (Haney and McDonald 1995). Less ice has been shown to translate into lower reproductive success in Ivory Gulls (Dalgety 1932) so they may be particularly sensitive to decreasing sea ice. Another possibility is that the decline could be due to excessive ice at the Ivory Gulls' wintering grounds, where sea ice has actually increased since the 1950s (Stern and Heide-Jorgensen 2003). Because the Ivory Gulls need a combination of ice and open water to access prey species, the near-total freeze-up could have resulted in decreased food availability (Krajick 2003).

1.6.3 Contamination

The Ivory Gull had the highest level of many chemicals including DDT, PCBs and HCH of any seabird in the Northwater Polynya, including the Glaucous Gull which feeds at a slightly higher trophic level (Fisk, Hobson and Norstrom 2001; Buckman *et al.* 2004). Ivory Gulls also had the highest mean value of total mercury concentration found in Canadian Arctic seabird eggs, 2.5 times higher than Glaucous Gulls, suggesting that factors other than the Ivory Gulls' trophic level are contributing to its mercury exposure (Braune, Mallory and Gilchrist 2006). The mercury concentrations present in the Ivory Gull eggs are at or above those that are known to have a detrimental effect on breeding success (Braune, Mallory and Gilchrist 2006).

One of the major conservation concerns with Ross's Gull is the potential for the

dense fall population in the Chukchi and western Beaufort Seas to be devastated by an oil spill or other pollution event (Alvo *et al.* 1996). Several Ivory Gulls that appeared in St. John's Newfoundland in the winter of 1997-1998 were oiled, indicating they are also vulnerable (Ian L. Jones personal communication).

1.6.4 Disturbance

Disturbance has been a significant problem for Ross's Gulls breeding near Churchill, resulting in several nest abandonments (Alvo *et al.* 1996). Ivory Gulls and Ross's Gulls are thought to purposely nest in remote places to avoid any disturbance and so are often very sensitive to it (Haney and MacDonald 1995; Alvo *et al.* 1996).

1.7 Thesis Outline and Objectives

This thesis is a study of the conservation genetics of the Ivory Gull and Ross's Gull. The main objectives were to quantify geographic patterns of mitochondrial genetic variation of both breeding colonies and wintering areas and to deduce the evolutionary history of these species.

In Chapter 2 "Conservation Genetics and Phylogeography of the Endangered Ivory Gull (*Pagophila eburnea*)" I describe the distribution of genetic variation in both control region sequences and combined mitochondrial gene sequences between breeding colonies and wintering areas. This information is considered necessary as this species has experienced strong declines in counts Canada over the past decade and so a management strategy is urgently needed. I also use the genetic diversity in this species to make inferences about the population history such as the long-term effective population size.

In Chapter 3 "Genetic Diversity and Differentiation of Ross's Gull (*Rhodostethia rosea*)" I describe the control region genetic differentiation between Canadian breeding birds and those wintering off Alaska. These data are essential because of the extremely low Canadian population size and the low productivity observed. I also compare the genetic diversity and assessed the population history of both populations.

In Chapter 4 "General Discussion, Future Directions and Recommendations" I review the conclusions of my study and the data it provides for avian conservation genetics. Further required research on Ivory Gulls and Ross's Gulls is summarized and policies needed to prevent the extinction of these species are outlined.

Literature Cited

Alvo, R., McRae, D., Holohan, S., Divoky, G. 1996. Updated Status Report on the Ross's Gull *Rhodostethia rosea* in Canada. Committee on the Status of Endangered Wildlife in Canada.

Amos, W. and Harwood, J. 1998. Factors affecting levels of genetic diversity in natural populations. London Royal Society 353: 177-186.

Anker-Nilssen, T., Bakken, V., Strøm, H., Golovkin, A., Bianki, V. and Tatarinkova, I. 2000. The Status of Marine Birds Breeding in the Barents Sea Region. Norsk Polarinstitutt Rapp. Ser. No. 113.

Ardern, S.L. and Lambert, D.M. 1997. Is the black robin in genetic peril? Molecular Ecology 6: 21-28.

Avise, J.C. 2004. Molecular Markers, Natural History, and Evolution. Sinauer Associates: Sunderland, MA. 684pp.

Avise, J.C. 1998. The history and purview of phylogeography: a personal reflection. Molecular Ecology 7: 371-379.

Avise, J.C. 1995. Mitochondrial DNA polymorphism and a connection between genetics and demography of relevance to conservation. Conservation Biology 9: 686-690.

Avise, J.C. and Walker, D. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the London Royal Society B. 265: 457-463.

Avise, J.C., Nelson, W., Bowen, B. and Walker, D. 2000. Phylogeography of colonial nesting seabirds, with special reference to global matrilineal patterns in the Sooty Tern (*Sterna fuscata*). Molecular Ecology 9: 1783-1792.

Ballard, J.W. and Whitlock, M.C. 2004. The incomplete natural history of mitochondria. Molecular Ecology 13: 729-744.

Bateson, P.P.G. and Plowright, R.C. 1959a. The breeding biology of the Ivory Gull in Spitsbergen. British Birds 52: 105-114.

Bechet, A., Martin, J., Meister, P. and Rabouam, C. 2000. A second breeding site for Ross's Gull (*Rhodostethia rosea*) in Nunavut, Canada. Arctic 53: 234-236.

Bensch S., Hasselquist, D. and Von Schantz, T. 1994. Genetic similarity between parents predicts hatching failure: Non-incestuous breeding in the great reed warbler. Evolution 48: 317-326.

Birkenmajer, K. 1969. Observations on Ivory Gull *Pagophila eburnea* (Phipps) in south Vestspitsbergen. Acta Ornitologica 11: 461-476.

Braune, B.M., Mallory, M.L. and Gilchrist, H.G. 2006. Elevated mercury levels in a declining population of Ivory Gulls in the Canadian Arctic. Marine Pollution Bulletin 52: 969-987.

Briskie, J.V. and Mackintosh, M. 2004. Hatching failure increases with severity of population bottlenecks in birds. Proceedings of National Academy of Sciences 101: 558-561.

Brown, W.M., George, M. and Wilson, A.C. 1979. Rapid evolution of animal mitochondrial DNA. Proceedings of National Academy of Sciences 76: 1967-1971.

Buckman, A. H., Norstrom, R., Hobson, K., Karnovsky, N., Duffe, J. and Fisk, A. 2004. Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. Environmental Pollution 128: 327-338.

Buturlin, S.A. 1906. The breeding-grounds of the Rosy Gull. Ibis 8: 131-139, 333-337, 661-666.

Chardine, J.W., Fontaine, A., Blokpoel, H., Mallory, M. and Hofmann, T.2004. At-sea observations of Ivory Gulls (*Pagophila eburnea*) in the eastern Canadian high Arctic in 1993 and 2002 indicate a population decline. Polar Record 40: 355-359.

Chartier, B. And Cooke, F. 1980. Ross's Gulls (*Rhodostethia rosea*) nesting at Churchill, Manitoba, Canada. American Birds 34: 839-841.

Chen, J.Z., Yauk, C., Herbert, C. and Herbert, P. 2001. Genetic Variation in Mitochondrial DNA of North American Herring Gulls, *Larus argentatus*. Journal of Great Lakes Research 27: 199-209.

Clayton, D.A. 1992. Structure and function of the mitochondrial genome. Journal of Inherited Metabolic Disease 15: 439-447.

Connor, J.K. and Hartl, D.L. 2004. A Primer of Ecological Genetics. Sinauer Associates: Sunderland MA. 304pp

Conservation of Arctic Flora and Fauna (CAFF). 2004. Arctic Flora and Fauna: Status and Conservation. 250pp.

Cooper, A. 1994. DNA from museum specimens. In: Herrmann B., Herrmann, S. (eds) Ancient DNA: recovery and analysis of genetic material from paleontological, archaeological, museum, medical and forensic specimens. Springer, New York.

COSEWIC. 2006. COSEWIC assessment and update status report on the Ivory Gull *Pagophila eburnea* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 42pp.

Cramp, S. and K.E.L. Simmons. 1983. Birds of the Western Palearctic. v.3. New York: Oxford University Press.

Crochet, P.A., Bonhomme, F. and Lebreton, J.D. 2000. Molecular phylogeny and plumage evolution in gulls (*Larini*). Journal of Evolutionary Biology 13: 47-57.

Crochet, P.A. and Desmarais, E. 2000. Slow rate of evolution in the mitochondrial control region of gulls (*Aves : Laridae*). Molecular Biology and Evolution 17: 1797-1806.

Crow, J.F. and Aoki, K. 1982. Group selection for a polygenic behavioural trait: a differential proliferation model. Proceedings of National Academy of Sciences 79: 2628-2631.

Cummings, M.P., Otto, S.P. and Wakeley, J. 1995. Sampling properties of DNA sequence in phylogenetic analysis. Molecular Biology and Evolution 12: 814-822.

Dalgety, C.T. 1932. The Ivory Gull in Spitsbergen. British Birds 26:2-7.

Degtyarev, A.G., Labutin, Y.V. and Blohin, Y.Y. 1987. Ross's Gull *Rhodostethia rosea*: Notes on its migration and on peculiarities in its reproductive cycle near the limit of its distribution. Zoological Journal 66: 1873-1885.

Densley, M. 1999. In Search of Ross's Gull. Peregrine Books. 268pp

Densley, M. 1979. Ross's Gulls in Alaska. British Birds 72: 23-28.

Divoky, G. 1976. The pelagic feeding habits of Ivory and Ross' Gulls. Condor 78: 85-90.

Dynesius, M. and Jansson, R. 2000. Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. Proceedings of National Academy of Sciences 97: 9115-9120.

Feldman, C.R. and Omland, K.E. 2005. Phylogenetics of the common raven complex (*Corvus*: Corvidae) and the utility of ND4, COI and intron 7 of the ß-fibrinogen gene in avian molecular systematics. Zoologica Scripta 34: 145-156.

Fisk, A., Hobson, K. and Norstrom, R. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya Marine Food Web. Environmental Science & Technology 35: 732-738.

Frankham, R., Ballou, J.D. and Briscoe, D.A. 2004. A Primer of Conservation Genetics. Cambridge University Press: United Kingdom. 220pp.

Frankham, R. and Ralls, K. 1998. Inbreeding leads to extinction. Nature 392: 441-442.

Frankham, R. 1998. Inbreeding and extinction: Island populations. Conservation Biology 12: 665-675.

Freeland, J.R., Anderson, S., Allen, D. and Looney, D. 2007. Museum samples provide novel insights into the taxonomy and genetic diversity of Irish Red Grouse. Conservation Genetics 8: 695-703.

Genovart, M., Oro, D. and Bonhomme, F. 2003. Genetic and morphological differentiation between the two largest breeding colonies of Audouin's Gull *Larus audouinii*. Ibis 145: 448-456.

Gilchrist, H.G. and Mallory, M. 2005. Declines in abundance and distribution of the Ivory Gull (*Pagophila eburnea*) in Arctic Canada. Biological Conservation 121: 303-309.

Glenn, T.C., Stephan, W. and Braun, M.J. 1999. Effects of a population bottleneck on Whooping Crane mitochondrial DNA variation. Conservation Biology 13: 1097-1107.

Haney, J.C. and MacDonald, S.D. 1995. Ivory Gull (*Pagophila eburnea*). In The Birds of North America, No. 175 (Poole, A. and Gill, F., eds). The Birds of North America, Inc., Philadelphia.

Haney, J.C. 1993. Rare, local, little known and declining North American breeders. A closer look: Ivory gull. Birding 24: 330-338.

Hayashi, J.I., Tagashira, Y. and Yoshida, M.C. 1985. Absence of extensive recombination between inter- and intraspecies mitochondrial DNA in mammalian cells. Experimental Cell Research 160: 387-395.

Hendrickson, S.L., Bleiweiss, R., Matheus, J., de Matheus, L., Jacome, N. and Pavez, E. 2003. Low genetic variability in the geographically widespread Andean Condor. Condor 105: 1-12.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnean Society 58: 247-276.

Hjort, C., Gudmundsson, G. and Elander, M. 1997. Ross's Gulls in the central Arctic Ocean. Arctic 50: 289-292.

Houde, P. *et al.* 1997. Phylogeny and Evolution of 12S rDNA in Gruiformes (Aves). In Avian Molecular Evolution and Systematics. (Mindell, D. ed.) p.121-158.

Jamieson, I.G., Ryan, C.J. 2000. Increased egg infertility associated with translocating inbred Takahe (*Porphyrio hochstetteri*) to island refuges in New Zealand. Biological Conservation 94: 107-114.

Kampp, K. and Kristensen, R.M. 1980. Ross's Gull *Rhodostethia rosea* breeding in Disko Bay, West Greenland, 1979. Dansk Ornithologisk Forenings Tidsskrift 74: 65-74.

Krajick, K. 2003. In search of the Ivory Gull. Science 301: 1840-1841.

Lansman, R.A., Avise, J.C. and Huettel, M.D. 1983. Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. Proceedings of National Academy of Science 80: 1969-1971.

Liebers, D. and Helbig, A.J. 2002. Phylogeography and colonization history of Lesser Black-backed Gulls (*Larus fuscus*) as revealed by mtDNA sequences. Journal of Evolutionary Biology 15: 1021-1033.

Liebers, D., Helbig, A.J. and De Kniff, P. 2001. Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* group (Aves: Charadriiformes). Molecular Ecology 10: 2447-2462.

McDonald, S.D. 1978. First breeding of Ross's Gull in Canada. Proceedings of the Colonial Waterbird Group. 1978: 16.

MacDonald, S.D. 1976. Phantoms of the polar pack-ice. Audubon 78: 2-19.

MacDonald, S.D. and MacPherson, A.H. 1962. Breeding places of the Ivory gull in Arctic Canada. National Museums of Canada Bulletin 183: 111-117.

Mallory, M.L., Gilchrist, H.G. and Mallory, C.L. 2006. Ross's Gull (*Rhodostethia rosea*) Breeding in Penny Strait, Nunavut, Canada. Arctic: 59: 319-321.

Mallory, M. and Gilchrist, G. 2005. Population trends and habitat requirements of Ivory Gulls breeding in Nunavut: Ivory Gull 2005 report. 13p.

Marshall, H.D. and Baker, A.J. 1997. Structural conservation and variation in the mitochondrial control region of Fringilline Finches (*Fringilla* spp.) and the Greenfinch (*Carduelis chloris*). Molecular Biology and Evolution 14: 173-184.

Martin, P.R. and McKay, J.K. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. Evolution 58: 938-945.

Meltofte, H., Edelstam, C., Granstrom, G., Hammer, H. and Hjort, C. 1981. Ross's gulls in the Arctic pack-ice. British Birds 74: 316-320.

Mila, B., Girman, D.J., Kimura, M. and Smith, T.B. 2000. Genetic evidence for the effect of the postglacial population expansion of the phylogeography of a north American Songbird. Proceedings of the Royal Society of London B. 267: 1033-1040.

Mindell, D.P. and Thacker, C.E. 1996. Rates of molecular evolution: Phylogenetic issues and applications. Annual Review of Ecology and Systematics 27: 279-303.

Moum, T. and Arnason, E. 2001. Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. Molecular Ecology 10: 2463-2478.

Murata, K., Satou, M., Matsushima, K., Satake, S. and Yamamoto, Y. 2004. Retrospective estimation of genetic diversity of an extinct Oriental White Stork *Ciconia*

boyciana population in Japan using mounted specimens and implications for reintroduction programs. Conservation Genetics 5: 553-560.

Nettleship, D.N. and Evans, P.G.H. 1985. Distribution and status of the Atlantic Alcidae. In: *The Atlantic Alcidae* (eds Nettleship, D.N. and Birkhead, T.R.), pp. 53-154. Academic Press, New York.

Newton, I. 2003. The Speciation and Biogeography of Birds. Academic Press, Amsterdam. 668pp.

Orr, C. and Parsons, J. 1982. Ivory Gulls, *Pagophila eburnea*, and ice edges in Davis Strait and the Labrador Sea. Canadian Field-Naturalist 96: 323-328.

Palumbi, S.R. and Baker, C.S. 1994. Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. Molecular Biology and Evolution 11: 426-435.

Patirana, A., Hatch, S.A. and Friesen, V.L. 2002. Population differentiation in the Red-legged Kittiwake (*Rissa brevirostris*) as revealed by mitochondrial DNA. Conservation Genetics 3: 335-340.

Patirana, A. 2000. Global population genetics and evolutionary history of Black-legged Kittiwakes (*Rissa tridactyla*) as revealed by mitochondrial and nuclear DNA. Unpublished M.Sc. thesis. Queen's University.

Payne, R.B. and Sorenson, M.D. 2002. Museum collections as sources of genetic data. In: R. van den Elzen (ed) Bird Collections in Europe: promotion of science in ornithology. Alexander Koenig Research Institute and Museum of Zoology Proceedings, Vol. 2, Bonn, Germany.

Pearce, J.M., Talbot, S.L., Petersen, M.R. and Rearick, J.R. 2005. Limited genetic differentiation among breeding, molting, and wintering groups of the threatened Steller's Eider: the role of historic and contemporary factors. Conservation Genetics 6: 743-757.

Pearce, J.M., Talbot, S., Pierson, B., Petersen, M., Scribner, K., Dickson, D.L. and Mosbech, A. 2004. Lack of spatial genetic structure among nesting and wintering King Eiders. Condor 106: 229-240.

Pons, J-M., Hassanin, A. and Crochet, P-A. 2005. Phylogenetic relationships within the *Laridae* (Charadriiformes: *Aves*) inferred from mitochondrial markers. Molecular Phylogenetics and Evolution 37: 686-699.

Porsild, A.E. 1943. Birds of the Mackenzie Delta. Canadian Field Naturalist 57: 19-35.

Randi, E. and Lucchini, V. 1998. Organization and evolution of the mitochondrial DNA control region in the avian genus *Alectoris*. Journal of Molecular Evolution 47: 449-462.

Reed, D.H. and Frankham, F. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17: 230-237.

Reed, D.H., Briscoe, D.A. and Frankham, F. 2002. Inbreeding and extinction: The effect of environmental stress and lineage. Conservation Genetics 3: 301-307.

Ross, J.D., Arndt, A., Smith, R., Johnson, J. and Bouzat, J. 2006. Re-examination of the historical range of the Greater Prairie Chicken using provenance data and DNA analysis of museum collections. Conservation Genetics 7: 735-751.

Ruokonen, M., Kvist, L., Aarvak, T., Markkola, J., Morozov, V., Oien, I., Syroechkovsky, E., Tolvanen, P. and Lumme, J. 2004. Population genetic structure and conservation of the Lesser White-fronted Goose *Anser erythropus*. Conservation Genetics 5: 501-512.

Ruokonen, M. and Kvist, L. 2002. Structure and evolution of the avian mitochondrial control region. Molecular Phylogenetics and Evolution 23: 422-432.

Ryder, O.A. 2005. Conservation genomics: applying whole genome studies to species conservation efforts. Cytogenetic and Genome Research 108: 6-15.

Saccheri, I., Kuussaari, M., Kankare, M., Vikman, P., Fortelius, W. and Hanski, I. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392: 491-494.

Sefc, K.M., Payne, R.B. and Sorenson, M.D. 2007. Single base errors in PCR products from avian museum specimens and their effect on estimates of historical genetic diversity. Conservation Genetics 8: 879-884.

Spielman, D., Brook, B.W. and Frankham, R. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences USA 101: 15261-15264.

Stenhouse, I.J., Robertson, G.J. and Gilchrist, H.G. 2004. Recoveries and survival rates of Ivory Gulls (*Pagophila eburnea*) banded in Nunavut, Canada, 1971-1999. Waterbirds 27: 486-492.

Stenhouse, I.J. 2003. Ivory Gull Conservation Strategy and Action Plan. Canadian Ivory Gull Working Group.

Stern, H.L. and Heide-Jorgensen, M.P. 2003. Trends and variability of sea ice in Baffin Bay and Davis Strait, 1953-2001. Polar Research 22: 11-18.

Tomkovich, P.S. 1990. On the post-fledging movements of Ivory Gulls (*Pagophila eburnea*). Modern Ornithology. 150-151.

Vallianatos, M., Lougheed, S.C. and Boag, P.T. 2002. Conservation genetics of the Loggerhead Shrike (*Lanius ludovicianus*) in central and eastern North America. Conservation Genetics 3: 1-13.

van der Kuyl, A.C., Kuiken, C., Dekker, J. and Goudsmit, J. 1995. Phylogeny of African monkeys based upon mitochondrial 12S rRNA sequences. Journal of Molecular Evolution 40: 173-180.

Volkov, A.E. and de Korte, J. 1996. Distribution and numbers of breeding Ivory Gulls *Pagophila eburnea* in Severnaja Zemlja, Russian Arctic. Polar Research 15: 11-21.

Vuilleumier, F. 1995. A large colony of Ivory Gulls (*Pagophila eburnea*) on Victoria Island, Russia. Alauda 63: 135-148.

Wilson, A.C., Cann, R., Carr, S., George, M., Gyllensten, U., Helm-Bychowski, K., Higuchi, R., Palumbi, S., Prager, E., Sage, R. and Stoneking, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of the Linnean Society 26: 375-400.

Zhang, B., Fang, S-G. and Xi, Y-M. Low genetic diversity in the endangered Crested Ibis *Nipponia nippon* and implications for conservation. Bird Conservation International 14: 183-190.

Zink, R.M., Rohwer, S., Andreev, A.V. and Dittmann, D.L. 1995. Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. Condor 97: 639-649.

Zubakin, V.A. 1984. Ivory Gull *In Red Data book of the USSR*, 2nd ed., A.M. Borodin *et al.* eds, Leshnaya Promishlennost, Moscow.

Zubakin, V.A. and Avdanin, V.O. 1983. Peculiarities of colonial nesting in *Rhodostethia rosea*. Zoologicheskij zhurnal 62: 1754-1756.

Chapter 2: Conservation Genetics and Phylogeography of the Endangered Ivory Gull (*Pagophila eburnea*)

Introduction

Counts of Ivory Gulls (*Pagophila eburnea*) at traditional Arctic Canadian breeding colony sites have declined by 80% over the past 15 years (Gilchrist and Mallory 2005). If this trend reflects real population decline, this would represent one of the most precipitous declines of any avian species ever detected in North America. The Canadian breeding population (representing all of North America's breeding Ivory Gulls) may have declined from an estimated 1200 pairs in the 1980s to as few as 250-350 pairs in 2005 (Gilchrist and Mallory 2005). As a result, Ivory Gulls were assessed as Endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2006. Both the population and distribution of Ivory Gulls in North America appear to have been shrinking since the late 1800s, and they now breed only on Ellesmere Island, Seymour Island and Brodeur Island, in northern Nunavut (Haney and McDonald 1995). The Canadian population is considered to represent 10-30% of the global population, which makes it significant to the species' survival (Gilchrist and Mallory 2005).

The last global population estimate, taken before the declines were observed in Canada, was 10,000 breeding birds (Vuillemier 1995). The actual count could be much lower if the declines in Canada are also occurring in other areas of the Arctic. Globally, the population status of the Ivory Gull is unknown (Krajick 2003), but it is classified as Declining in Svalbard (Anker-Nilssen *et al.* 2000) and Rare in Russia (Zubakin 1984).

Although several thousand birds were estimated to breed in Franz Josef Land, Russia at the turn of the century (Haney and McDonald 1995), no colonies were found in a 1996 survey of previously identified nesting sites in a major breeding region (Krajick 2003). Ivory Gulls have declined in Spitsbergen since the nineteenth century, when colonies of 100 or more pairs were often recorded (Bateson and Plowright 1959). The last population estimates made in the 1960s found only 344 pairs (Birkenmajer 1969). More than 200 birds were banded in summer 2003 in Greenland, but many areas of Greenland are not well explored, so the suggested stable population size of 1,000 birds could be inaccurate (Krajick 2003).

Band recoveries indicate that Ivory Gulls are capable of moving long distances, for example 2,700 miles from Franz Josef Land, Russia to Labrador, Canada (Tuck 1971). A bird banded during the breeding season in Greenland was recovered three years later during the breeding season in Franz Josef Land, suggesting that they may change breeding regions (Salomonsen 1979). Unfortunately, the total number of banding returns is too small to indicate how general this movement might be (Cramp and Simmons 1983). After the June-September breeding season, Ivory Gulls spend the winter in two main areas: in the Labrador Sea stretching from the shores of Newfoundland and Labrador, to Greenland and in the northern Bering Sea off western Alaska (Haney and McDonald 1995).

There is little detailed information available about both the global and colonial population structure of Ivory Gulls. Since many aspects of behavior, such as dispersal, degree of philopatry and duration of pair bonds, play significant roles in how genetic variation is structured within and among avian populations (Chesser 1991a, b),

determination of the geographic partitioning of genetic variance will help to provide some clues about Ivory Gull population structure. Information about the evolutionary history and genetic structure are critical for the success of conservation programmes, because these data permit definition of management units and the design of strategies aimed at preserving genetic variation (Haig 1998). Understanding the population structure of Ivory Gulls will also help determine which breeding areas are affected by hunting during the non-breeding season in Greenland and eastern Russia (Stenhouse, Robertson and Gilchrist 2004).

As a result of harsh climate fluctuations, arctic species tend to experience more frequent and severe population bottlenecks than temperate species (Dynesius and Jansson 2000). The small population size associated with isolation in refugia and the repeated withdrawal from and recolonization of their ranges result in low genetic diversity in arctic species (Hewitt 1996; Martin and McKay 2004). There is considerable variation among species in the degree of phylogeographic structure as a result of unique life-history traits and the degree of fragmentation into refugia during ice ages such as the Pleistocene glaciation (Avise and Walker 1998). Ivory Gulls have a holarctic distribution (Haney and McDonald 1995), which suggests that they may have survived in multiple refugia. Alternatively, Ivory Gulls may have persisted in one area and then expanded to other arctic areas. There are examples of both of these scenarios in circumpolar arctic birds. Thick-billed Murres (*Uria lomvia*) have strongly differentiated Atlantic and Pacific clades (Birt-Friesen *et al.* 1992), which indicates that they survived severe glacial periods in separate refugia. In contrast, species such as the King Eider (*Somateria spectabilis*) show

little population genetic structure despite distinct populations with different wintering areas, which suggests expansion from a single refugium (Pearce *et al.* 2004).

Ivory Gulls are the sole members of the genus *Pagophila* and exhibit distinctive behavioral and ecological differences from other gulls (Haney and McDonald 1995). The only previous genetic work on Ivory Gulls was a component of a phylogenetic study of 32 (Crochet, Bonhomme and Lebreton 2000) and 53 gull species (Pons, Hassanin and Crochet 2005), which demonstrated that Ivory Gulls are most closely related to Sabine's Gull (*Xema sabini*) and that kittiwakes (*Rissa* spp.) are their next closest relatives. Black-legged Kittiwakes exhibit strong genetic population structure as Pacific and Atlantic populations are significantly different from each other and there are several differentiated populations in the North Atlantic, including Newfoundland (Patirana 2000). Red-legged Kittiwakes, which breed only on islands in the Bering Sea off Alaska and are of conservation concern, showed some population structure, likely a result of their strong nesting site fidelity (Patirana, Hatch and Friesen 2002). However, Auduoin's Gull, which is also of conservation concern, showed no genetic variation in the mtDNA Control Region among any of the 16 individuals sampled, despite being philopatric and having different body sizes at different colonies (Genovart, Oro and Bonhomme 2003).

The loss of genetic diversity is always a concern with endangered species, and they often have lower genetic variation than related, unthreatened species (Spielman, Brook and Frankham 2004). The decline of populations often results in loss of rare alleles and decreased heterozygosity, which can theoretically affect the ability of the species to persist and adapt in the face of environmental change (Frankham and Ralls 1998; Reed, Briscoe and Frankham 2002). This theory is based on two arguments: the

requirement of genetic diversity for evolution to occur and the expected positive relationship between heterozygosity and population fitness (Reed and Frankham 2003). Genetic diversity is a key parameter of a populations' likelihood of recovery, and low genetic diversity carries with it an increased risk of extinction, especially during times of environmental stress (Frankham 1998; Frankham and Ralls 1998; Reed, Briscoe and Frankham 2002). Major environmental threats to Ivory Gulls include high levels of organochlorine contamination (Fisk, Hobson and Norstrom 2001; Buckman *et al.* 2004) including mercury (Braune, Mallory and Gilchrist 2006) and the negative effect of global warming (Dalgety 1932). However, several authors have questioned the evidence for the deleterious effects of loss of genetic diversity, pointing to the existence of viable populations of numerous species in the absence of genetic diversity due to being bottlenecked to only a few individuals (e.g. Black Robin *Petroica traversi* (Arden and Lambert 1997), Whooping Crane *Grus americana* (Glenn, Stephan and Braun 1999), and Crested Ibis *Nipponia nippon* (Zhang, Fang and Xi 2004)).

I used museum specimens to determine mitochondrial sequences of Ivory Gulls. Museum specimens have been used in previous avian conservation genetic studies (e.g. Loggerhead Shrike *Lanius ludovicianus* (Vallianatos, Lougheed and Boag 2002); Oriental White Stork *Ciconia boyciana* (Murata *et al.* 2004); and Heath Hen *Tympanuchus cupido* (Ross *et al.* 2006)). This was however, the first time that a genetic study of several genes was performed solely with avian museum specimens. Mitochondrial sequences are present in much higher copy number than nuclear genes (Ballard and Whitlock 2004) and since some of the samples were as old as 100 years, the probability of intact sequences is higher for mtDNA (Cooper 1994). As well, due to the maternal inheritance of mtDNA,

its theoretical effective population size is ¼ that of nuclear markers and it should therefore reach equilibrium sooner (Wilson *et al.* 1985). Several gene regions, including the control region, were used to increase the chance of finding polymorphisms.

My aim in this study was to use mitochondrial DNA sequencing to understand the circumpolar genetic structure of the Ivory Gulls. If Ivory Gulls are distributed across several genetically distinct breeding populations, these might require separate management. Alternatively, if Ivory Gulls represent a panmictic population united by high gene flow between the various colonies despite large distances between them perhaps joint management as a single unit would be more appropriate and this would allow for the potential of translocation if the declines seen in Canada are not happening elsewhere. The origin of birds that winter in the Labrador Sea and Alaska areas was also investigated. The two wintering areas were also compared to determine whether potential genetic differentiation in Ivory Gulls is more influenced by wintering areas than by breeding areas. Information regarding the wintering area is especially important as the decline in numbers of Ivory Gulls seen in Canada has been suggested to be a result of factors related to the wintering grounds or migration from the wintering grounds (Gilchrist and Mallory 2005). The extensive use of museum specimens allowed the analysis of population genetic parameters through time. I also tried to understand the population history by inferring evidence for population bottlenecks and the long-term effective population size.

Materials and Methods

Samples

A total of 126 Ivory Gulls were analyzed using several different mitochondrial DNA markers (Table 2.3.1; Appendix 1). Individuals were designated as 'breeding' if the specimen was taken during the summer breeding season (June until early September (Haney and McDonald 1995)) at a potential breeding colony. Individuals were designated as non-breeding if the specimen was taken at another time of year or at a non-breeding location (for example, Ivory Gulls do not breed in Alaska (Haney and McDonald 1995) so even if the bird was taken during the summer it would not be counted as a breeding bird).

DNA extraction

A small (~1mm^2) piece of tissue was cut from either the toe pad or skin with a sterile razor blade in a sterile weigh boat. To avoid contamination, a new sterile razor blade and weigh boat were used and both the bench and my gloves were wiped clean with 75% ethanol before each new sample. DNA was extracted from the tissue with the QIAamp DNA Mini Kit Tissue Protocol (Qiagen Inc.). Tissue samples were digested in 180µL of Buffer ATL (Qiagen Inc.) with 20µL of proteinase K and incubated at 56°C overnight or until all of the tissue was completely lysed. The tube was removed and 200µL of Buffer AL (Qiagen Inc.) was added, mixed and incubated at 70°C for 10 minutes. Next, 200µL of 100% ethanol was added to the mixture and vortexed. The solution was applied to a QIAamp Spin Column and centrifuged for 1 minute. The filtrate was discarded, a clean collection tube was used and 500µL of Buffer AW1 was added to the spin column. The column was centrifuged for one minute and the filtrate

was discarded again. A new collection tube was used and 500µL of Buffer AW2 was added. The column was centrifuged for three minutes. The filtrate was removed and the column was centrifuged for an additional one minute. The collection tube was discarded and a clean 1.5mL tube was used. The DNA was redissolved by adding 200µL of distilled water to the spin column and incubating it for one minute at room temperature. The column was centrifuged for one minute before adding another 200µL of distilled water. The spin column was then centrifuged for one minute and the 1.5mL tube was removed with the filtrate, labeled and put in the freezer.

PCR amplification

Gull-specific oligonucleotide primers were designed for the Control Region (CR), 12S rRNA, ND4 and ND4L loci (Table 2.2.1). The CR primers were designed from the Ivory Gull CR sequences reported by Crochet, Bonhomme and Lebreton (2000). The entire mtDNA genome sequence of the Kelp Gull (*Larus dominicanus*) (obtained by Slack *et al.* 2007) (GenBank accession NC_007006) was used as a template for the 12S rRNA, ND4 and ND4L sequences.

A PCR cocktail was prepared using 10µL of dH_2O, 2.5µL of 10xPCR buffer, 0.5µL of dNTPs [20mM], 0.5µL of each primer [10mM] and 0.2µL (1 U) of Hot Start Taq polymerase. The sample tubes were composed of 15µL of PCR cocktail and 10µL of DNA. A control sample in which no DNA was added was always used to ensure that the cocktail didn't contain any DNA contamination. These samples were placed in an Eppendorf Mastercycler, and PCR amplification proceeded with a specific program according to the primer pair. Each program started with 15 minutes at 95°C to activate

the Taq polymerase activity. The PCR amplification cycle consisted of an initial denaturation stage of 45 seconds at 93 °C, the annealing stage which was different with each primer pair (see Table 2.2.1 for temperature and time), and then elongation for one minute at 72 °C. This cycle was repeated 45 times to ensure adequate DNA amplification. After the last cycle, a final amplification at 72 °C for five minutes was performed, and then the samples were held at 5 °C.

The samples were run on a 2% agarose gel for approximately 25 minutes to determine if a product was present. A ladder was used as the first lane and then each lane was comprised of 4μL DNA and 2μL dye. The gels were checked under UV and a photo was taken. If a band was present in the control sample then the samples were discarded. If no control sample band was present then samples that produced a band would continue to the PCR cleanup stage.

PCR cleanup

To remove unincorporated nucleotide and other PCR components, 5 volumes (105 μL) of QIAquick Buffer PB was added to the PCR sample and vortexed. The solution was transferred to a QIAquick spin column in 2mL collection tube. The column was centrifuged for one minute and the filtrate was discarded. The next step was the addition of 750μL of Buffer PE to the spin column and the column was again centrifuged for one minute. The filtrate was removed and the spin column was centrifuged for an additional minute. The collection tube was then replaced with a 1.5mL tube. Lastly, 30μL of distilled water was added to the spin column and it was left to stand for one minute. The column was then centrifuged for one minute and the filtrate was kept, labeled and frozen.

Sequencing reaction

Both forward and reverse sequencing reactions were done. Tubes were labeled and 5μL of DNA was added to each of the samples' corresponding forward and reverse tubes. The tubes were vacufuged for 10 minutes or until there was no longer any liquid. The cocktail was then prepared using 3.48μL of distilled water, 2μL of Big Dye and 0.32μL of either the forward or reverse primer per sample. Next, 5.8μL of the cocktail was added to each sample and the tube was vortexed, spun and put in the Eppendorf Mastercycler. Reactions were carried out with an initial two minute separation stage at 96°C before beginning the cycle of 0:30 at 96°C, 0:15 at 50°C and 4:00 at 60°C. This cycle was repeated 45 times and then the samples were held at 5°C.

Sequencing cleanup

This stage began with the addition of 40μL of 75% isopropanol. The solution was then vortexed and left to precipitate, with the caps off under a Kimwipe, for over 20 minutes. The tubes were closed and put in the centrifuge for 20 minutes at 13000rpm. The supernatant was aspirated and 250μL of 75% isopropanol was then added, vortexed and left to precipitate with the caps off under a Kimwipe for over 10 minutes. The tubes were then closed and put in the centrifuge for 10 minutes at 13000rpm. The supernatant was aspirated and any remaining liquid was removed by placing the tubes in the vacufuge for 10 minutes. The dried samples were removed from the vacufuge and 5μL of foramide EDTA was added to the tubes and vortexed. The reactions were denatured in the Eppendorf Mastercycler by heating the samples up to 95°C for two minutes and then

reducing the temperature rapidly to 5°C until they were removed. The samples were vortexed again before either being added to the comb or placed in the fridge.

Automated DNA Sequencing

The samples were sequenced on an acrylamide gel using a 96 lane ABI 377 Sequencer. A 48 or 64 comb was used with each lane containing 1μL or 0.8μL of the cleaned up sample. The acrylamide gel was prepared using 8.0g of urea, 12.5mL of dH_20, 2.5mL of 10x TBE, 3.0mL of PAGE-PLUS, 125μL of ammonium persulfate (APS) and 12.5μL of TEMED. The plates were carefully cleaned prior to pouring and the gel was left to polymerize for at least an hour. A plate check was always run before the actual sequencing run to verify that the gel was suitable for sequencing. After the plate check, the comb was added, TBE was poured into the upper and lower wells and the heating block was attached. The sequencing run was started for two minutes and then the comb was removed. The top was placed on the upper chamber and the run was continued until it finished approximately eight hours later. The data was automatically transferred into a Gel File which was carefully tracked before export into Sequencher.

Analysis

Sequences were aligned and Single Nucleotide Polymorphisms (SNPs) were identified with Sequencher. To exclude the possibility of nuclear pseudogenes, I verified that amplified sequences were of mtDNA origin by comparing sequences obtained from skin, toe pad and liver and by comparing homologous sequences for other gulls and the published Ivory Gull control region sequence (Crochet, Bonhomme and Lebreton 2000).

Since the statistical software packages used were not able to incorporate deletions into the calculations, each deletion was changed to a SNP before importing the data into the program. The number of haplotypes, overall and regional haplotype diversities (Hd), overall nucleotide diversity (π) and the average number of differences between sequences (k) were obtained using DNASP version 4.0 (Rozas and Rozas 1999). Tajima's D (Tajima 1989) and Fu's F-statistics (Fu 1997) were calculated for each separate population using ARLEQUIN version 3.0 (Excoffier, Laval and Schneider 2005) and overall using DNASP.

Analyses of molecular variance (AMOVA) were performed with ARLEQUIN to determine the proportion of total genetic variance represented at different hierarchical levels based on the geographical distribution of haplotypes, and the pairwise distances between them. AMOVA analyses were performed both on control region data and the collated sequences and several different groupings were tested. The results are reported as a series of hierarchal Φ statistics, which are analogous to F-statistics for diploid loci (Wright 1951). The value for Φ_{ST} reflects the structure among populations, the Φ_{SC} value reflects the structure among populations within groups and the Φ_{CT} value reflects the structure among groups (Excoffier, Smouse and Quattro 1992). The value for Φ_{ST} can also be defined as the correlation of random haplotypes within a population relative to haplotypes drawn from the entire sample (Excoffier, Smouse and Quattro 1992).

Based on pairwise Φ_{ST} values, each population was compared with all of the other populations to determine the degree of genetic differentiation among populations. Pairwise Φ_{ST} values were calculated with ARLEQUIN from the matrix of distances

between haplotypes. Pairwise Φ_{ST} analysis was done with both control region data and overall data.

To assess potential temporal differences in the genetic diversity of Ivory Gulls, I combined the samples into two groups: pre-1950 (N=62) and post-1950 (N=52). This allowed comparison of the genetic diversity parameters and the testing of the hypothesis that the genetic diversity has declined over time.

The long-term effective population size was estimated with the formula $N_e = 10^6(\pi/s)/g$, where π = the nucleotide diversity, s = rate of sequence divergence and g = the average generation time (Wilson *et al.* 1985). I used the rate of sequence divergence from the Lesser Black-backed Gull (*Larus fuscus*) control region data of 8.5% per million years (Liebers and Helbig 2002). The average generation time (g) was estimated as 10 years, because although Ivory Gulls can reproduce at the age of two they have a low reproductive success rate (Haney and McDonald 1995). Based on band returns, Stenhouse *et al.* (2004) suggest an average adult life expectancy of 6.4 ± 1.4 years, much lower than the 12-17 years that was previously estimated (Haney and McDonald 1995). An average generation time of 20 years was also used, since that was the value estimated for both Black-legged and Red-legged Kittiwakes, which are closely related and have similar life-histories (Patirana 2000; Patirana, Hatch and Friesen 2002).

I also compared the genetic diversity values for each mitochondrial marker sequenced to better characterize each gene region in Ivory Gulls. These are the first values obtained for the 12S rRNA, ND4 and ND4L loci in Laridae.

Results

Control Region sequence variation

I determined the sequence of a 264-bp segment of Domain I of the Control Region for 126 individuals representing three breeding areas (Canada, Greenland and Norway) and three wintering areas (Canada, Greenland and Alaska). These sequences contained six polymorphic positions (2.30% of the control region sequenced), three of which were parsimony-informative (1.10%). One of the most polymorphic parsimony informative sites was a single-base deletion in a sequence of 10 T's (H3). Similar Indels have been found to be associated with poly T or poly A portions of the control region in other species (Pearce 2006). The other major parsimony informative site was a G ↔ A transition at base 232 (H2). The third site was a C ↔ T transition seen in three individuals. The other polymorphic sites included two transversions (T ↔ A and A ↔ C) and one transition (T ↔ C).

These polymorphic sites defined seven unique haplotypes, three of which were only seen in one individual and one of which was only seen in two individuals. Haplotypes 1 and 2 were seen in all of the populations and Haplotype 3 was seen in four of the six populations, with the majority of the Haplotype 3 individuals from the Alaskan Non-Breeding group (see Table 2.3.1). Over 70% of the individuals sequenced were Haplotype 1 and all of the other haplotypes were either one or two base pair different (See Figure 2.3.2 for minimum spanning network).

The overall haplotypic diversity (Hd) was 0.451 and the nucleotide diversity (π) was 0.00207. Nucleotide diversity (π) was highest in the eastern areas, Norway and Alaska (both 0.00256) and lowest in the Greenland breeding birds (0.000510).

Haplotypic diversity was highest in the Norwegian and Alaskan birds and lowest in the Greenland breeding birds (Table 2.3.2).

Geographic structure of Control Region genetic variation

More than 0.900 of the genetic variance was found within each population (Table 2.3.4). Given a scenario in which no groups were identified *a priori* and each population was considered separate, approximately 0.957 of genetic variance was within the populations and only 0.0427 was seen between populations (p=0.0284). When grouped into western and eastern breeding populations, 0.0851 of the variance was found between the groups. Canada and Greenland breeding colonies were grouped together as they are very close geographically and this combination was supported with the extremely low value of variance found within the group (-0.0350 or almost zero). Although no Russian breeding birds were available, one hypothesis is that the Alaskan birds may represent Russian breeding birds. When the Alaskan birds were grouped with the Norwegian breeding population to form an eastern breeding group, the among-group variance was 0.0262 and the within group variance was 0.0405 but the within population variance remained similar. The fourth scenario, using Alaska to represent Russia, compared three breeding groups: Canada and Greenland, Norway, and Russia. When the Alaskan/Russian population was separated, among group variance was increased to 0.110 and the within group variance was almost nil (-0.0364). When the two wintering areas (Canada Non-Breeding and Greenland Non-Breeding representing the Labrador Sea area and the Alaska Non-Breeding area representing the Bering Sea) were compared the

among-group variance was 0.136. The Canada and Greenland non-breeding areas are very similar as the within group variance was almost zero (-0.0414).

The population structure was further investigated using pair-wise Φ_{ST} values to determine the level of differentiation between populations. The only significant differences were between the Alaskan non-breeding birds and the Canadian and Greenland breeding birds and the Canadian and Greenland non-breeding birds (p-values ranging from 0.0210 to 0.0490). Norwegian birds and the Alaskan non-breeding birds were not significantly different ($p = 0.0640$). None of the breeding colonies were significantly different from the others ($0.179 \leq p \leq 0.999$). The Canadian and Greenland non-breeding birds were not significantly different from each other ($p=0.894$), or from the breeding colonies ($0.217 \leq p \leq 0.999$). Taken together, the data indicate that Ivory Gulls have weak population structure, with little genetic variance that is found mostly within populations.

Population History

The overall Tajima's D was -1.10 and Fu's F was -1.89, neither of which were statistically significant (Table 2.3.5). When analyzed by population, the Canadian breeding population had a significant Fu's F = -1.60 ($p \leq 0.0450$) but the Tajima's D = -1.54 was not significant ($p \leq 0.0560$). All neutrality test values were negative, which suggests that the population is expanding from a historical bottleneck or that selection is occurring.

The long-term effective population size based on the observed nucleotide diversity of 2.07×10^{-3}, a generation time of 10 years and a sequence divergence rate of 8.5% per

million years (*Larus fuscus*; Liebers and Helbig 2002) was 2.4×10^3 female birds. On the assumption that Ivory Gulls are monogamous (Haney and McDonald 1995) and therefore have a sex ratio of approximately 1:1, the effective population size of the species is approximately 5000 birds. If an average generation of 20 years is assumed, the effective population size is approximately 2400 birds.

The hypothesis that the genetic diversity has declined in Ivory Gulls was tested by dividing the samples into pre- and post-1950 samples. Birds collected after 1950 (π = 0.00192, Hd = 0.413, k = 0.531) have lower genetic diversity than those collected prior to 1950 (π = 0.00233, Hd = 0.524, k = 0.614). When grouped as separate populations and tested using AMOVA, only 0.0218 of the variance was between populations (p=0.088).

Genomic Diversity

The Control Region had the highest nucleotide and haplotype diversity, but other gene regions provided parsimony-informative SNPs, especially 12S rRNA, which contained four. One of these (a transition from T ↔ C) was seen in 13 individuals, 12 of which also had the G ↔ A transition in the control region. All other SNPs found in the 12S rRNA, ND4-1 and ND4L regions were seen in only one or two individuals. The haplotype diversities of ND4-1 and ND4L were very low as a result of the small number of individuals with different haplotypes (0.101 and 0.121). If sequences other than the control region (as it was deemed essential) were not available, I reconstructed the complete haplotype on the assumption that the missing sequence was that of the common sequence. The exception to this was the three individuals with the CR 'A' haplotype, for which the 12S rRNA haplotypes were unknown. These individuals could have either the

12S rRNA 'C' or 'T' (the known ratio was 8:5) haplotypes, so calculations were performed with both alternatives. The reconstruction of missing data using the common haplotype may have resulted in an underestimation of the overall genetic diversity.

Genomic Geographic Structure

Of the three breeding colonies, Norway (k = 1.54) was approximately twice as variable as Canada (k= 0.886) and Greenland (k= 0.667) (Table 2.3.8). The diversities of the non-breeding populations were intermediate between that of the Canadian and Norwegian breeding colonies with the Greenland Non-Breeding having the highest nucleotide diversity and the Alaskan population having the highest haplotype diversity. The diversity of the Alaskan population is likely underestimated as it had the most missing sections, especially in ND4-1 (Appendix 1).

Most genetic variance (>0.950) was within the Ivory Gull populations with very little among groups or populations (Table 2.3.9). When no groups were assigned, the percentage of variance within populations was 0.959-0.969, which was significant (p=0.0108-0.0362). The largest among group variance (0.0705-0.0895) was between the Canada and Greenland non-breeding group and the Alaska non-breeding group but this was not significant (p=0.333-0.352). When the population was divided into eastern and western breeding populations (Norway representing the East and Canada and Greenland combined representing the West), among group variance was 0.0531-0.0655 (p=0.329-0.335) indicating no support for population structure. Alaska was the most differentiated as when it was added to the Eastern population to test the hypothesis that the Alaskan birds represent the Russian birds, the among group variance fell to -0.00780-0.00510

while the among population variance rose to 0.0375-0.0619 (from approximately negative one in the previous analysis) (p=0.00980-0.0567).

When the sequences were further analyzed by population pair-wise Φ_{ST} it became clear that Alaska was a distinct population, as it was statistically significantly different (p ranging from 0.006-0.0380 from each of the other populations, with the possible exception of Canada Breeding (p = 0.0180-0.0720) and Greenland Non-Breeding (p = 0.0270-0.0520). The Norwegian and Greenland Breeding populations were also weakly significantly differentiated in the genomic analysis (p= 0.0330-0.0580) but not in the control region analysis.

Genomic Population History

Tajima's D and Fu's F neutrality tests supported the hypothesis that some Ivory Gulls populations are expanding from population bottleneck. The Canadian Breeding population was the only population that was significantly negative for both tests (p = 0.0110-0.0200 and 0.0360-0.0560). The other populations were generally significant for one of the tests but not the other. For example, the Greenland Breeding population was significant for the Tajima's D test (p-value 0.00500-0.0150) whereas Fu's F-test actually gave a positive value indicating that the population is in equilibrium and not expanding. In the cases of the Norwegian Breeding, Canadian Non-Breeding and Alaskan Non-Breeding populations Fu's F test was significant and not Tajima's D. Finally, in the case of the Greenland Non-Breeding birds neither test was significant.

Discussion

Ivory Gull Genomic Diversity

The control region had the highest level of nucleotide and haplotype diversity (see Table 2.3.6) which is expected, as it is typically regarded as the most variable (Baker and Marshall 1997; Randi and Lucchini 1998). Domain II and especially Domain III of the control region have been shown to have unusually slow rates of sequence evolution in Laridae (Crochet and Desmarais 2000). As a result, Domain I was used, which generally exhibits high levels of size and sequence variation in birds (Marshall and Baker 1997), including the Herring Gull complex (*Larus cachinnans-fuscus*) (Liebers, Helbig and De Kniff 2001). The inclusion of the indel in the calculations is supported by research by Pearce (2006), who demonstrated that indels provide an important part of sequence divergence. The difference in the number of T's seen is consistent with the indel pattern seen previously and is likely due to the characteristics of poly T repetitive sequences such as slipped-strand mispairing, secondary structure or illegitimate elongation (Pearce 2006). The nucleotide diversity found in Ivory Gulls of 0.00207 is much lower than that of Red-legged Kittiwake at 0.015 (Patirana, Hatch and Friesen 2002).

The next most diverse region was ND4-3, which had a nucleotide diversity value of 0.000700 and haplotype diversity value of 0.405 as it contained five parsimony-informative SNPs. The ND4 gene had a much lower diversity in its first section as the ND4-1 region had the lowest haplotype diversity at 0.100. The region did have seven SNPs but all of these SNPs were seen in only one-two individuals. The ND4L region had the lowest nucleotide diversity at 0.000300 and only contributed one parsimony-informative SNP, seen in two individuals.

The 12S rRNA region had low nucleotide diversity but contained a highly polymorphic SNP that was seen in thirteen individuals. The nucleotide diversity value of 0.000400 was similar to that of the only published avian 12S rRNA analysis, the Andean Condor ($\pi = 0.0006$), and the haplotype diversity values were almost identical at 0.254 for the Ivory Gull and 0.25 for the Andean Condor (Hendrickson *et al.* 2003).

Ivory Gull Phylogeography

Less than 0.05 of the genetic variance in control region sequences occurred among groups of populations of Ivory Gulls. This is very low relative to other seabirds such as the Black Guillemot (*Cepphus grylle*) with 0.25 (Kidd and Friesen 1998) and the Sooty Tern (*Sterna fuscata*) with 0.38 (Avise *et al.* 2000). This is also low with respect to other gull species. Liebers, Helbig and De Kniff (2001) found that some 0.82 of total molecular variance was partitioned among the six Herring Gull taxa or groups of taxa. However, the fraction of genetic variance among groups was lower in Lesser Black-backed Gulls at 0.210 (Liebers and Helbig 2002). Black-legged Kittiwakes had 0.626 of their total genetic variance distributed among Atlantic and Pacific groups (Patirana 2000). However, when the Pacific and Atlantic colonies were assessed separately, significant genetic structuring was still seen in the Atlantic whereas the Pacific colonies had only 0.040 of the genetic variance between colonies (Patirana 2000).

Further analysis of the control region sequences with pair-wise Φ_{ST} indicated that the three breeding colonies of Ivory Gulls are genetically indistinguishable from each other ($p = 0.179$ between Norway and Greenland, 0.218 between Norway and Canada and 0.99 between Canada and Greenland). This suggests that these breeding birds may represent a

single management unit. The Canadian and Greenland Breeding birds are also very similar in AMOVA analysis. This similarity is shown when they are the only two populations combined into a group (in model 2 and model 4) which resulted in among population variance being a negative number and the F_{SC} being 1.00. The breeding colonies are also not differentiated from the Canadian and Greenland Non-Breeding birds with ($p = 0.217 - 0.999$). The Canadian and Greenland Non-Breeding birds are also not significantly differentiated from each other, consistent with the suggestion that the Labrador Sea birds are a single wintering population. Band recovery data support gene flow between breeding colonies as several long-distance movements have been reported. These include a bird banded in Franz Josef Land recovered in Labrador (Tuck 1971), a bird banded on Victoria Island recovered on the Kanin Peninsula (Anker-Nilssen 2000), and two birds banded in Greenland that were recovered in Franz Josef Land and south of Bjornoya (Salomonsen 1979). As well, populations that breed on flat land (instead of nunataks) often move from site to site (de Korte and Volkov 1993). The fidelity of Ivory Gulls to the breeding site is unknown but at least some marked individuals return to the same breeding colony from one year to the next (McDonald 1976).

In contrast to the other populations, the Alaskan Non-Breeding birds were found to be significantly differentiated from other Ivory Gull populations. Using AMOVA analysis, the among group variance increased to 0.109 (model 4) from 0.0262 (model 3) when the Alaskan population was considered a separate group. The largest among group variance (0.135) is seen when the Alaskan population is compared to the group of the Canadian and Greenland Non-Breeding birds. When the Alaskan population differentiation was further analyzed using pair-wise Φ_{ST}, statistically significant differentiation was

obtained when compared to all of the populations (Canada Breeding p=0.0430, Greenland Breeding p= 0.0430, Canada Non-Breeding p= 0.0210, Greenland Non-Breeding p= 0.0490) with the exception of the Norwegian Breeding birds where the p-value was 0.0640.

There are several hypotheses to account for why the Alaskan population is distinct from the other populations. The first possibility is that the Alaskan birds derive from Russian breeding colonies, and that Russian birds are genetically distinct from the other breeding colonies. The largest population of Ivory Gulls is thought to be in Russia, with a previous estimate of 10,000 breeding birds (Haney and McDonald 1995). Banding recoveries suggest that some of the Barents Sea population (which includes Russian birds), winter in the Bering Sea (Tomkovich 1990). There are previous observations by Hjort (1976) that Ivory Gulls migrate southwestwards along the East Greenland Current but these birds could have been from Norwegian breeding colonies. In North America, circumpolar movements are mostly from west to east (Renaud and McLaren 1982) but there are occasional sightings of Ivory Gulls during the fall near Tuktoyaktuk, Northwest Territories (Porsild 1943) which may be individuals moving southwest from the Canadian Arctic into the Bering Sea (Renaud and McLaren 1982). In the absence of any Russian birds in my sample, this hypothesis cannot be tested.

A second possibility is that Ivory Gulls are not breeding-site philopatric, but are instead wintering-site philopatric, and it is therefore the wintering sites that are distinct. The level of wintering site fidelity is unknown (McDonald and Haney 1995), but pair formation may take place before arrival at the breeding sites, as courtship displays have been recorded in offshore areas of the Chukchi Sea during early June (Kosygin as quoted in Ilyichev and Flint 1988). The population size of the Alaskan wintering birds is thought to

be much smaller than the main wintering population of the Labrador Sea (Orr and Parsons 1982), and they might appear in small numbers in the breeding colonies. This theory is supported by the occurrence of Haplotype 3 once in each of the Canadian and Norwegian breeding populations and once in the Newfoundland wintering population.

A third explanation is temporal. Since most of the Alaskan specimens are older, from the 1920s and 1930s, they may represent a colony that has since severely declined.

Ivory Gull Conservation Genomics

The overall level of genetic diversity in Ivory Gulls is low and similar to other endangered and arctic birds (see Table 2.4.1). One explanation for the low diversity is that Ivory Gulls experienced one or more severe historical population bottlenecks. Both Tajima's D and Fu's F tests were negative, although these values were not significant. The low genetic diversity is also consistent with the idea that Ivory Gulls are a panmictic population, as interbreeding populations may slowly lose genetic variation (Lacy 1987). In contrast, strongly subdivided populations often retain their genetic variation, resulting in a higher level of overall genetic diversity than interbreeding populations of equal size (Lacy 1987). Another possibility is that Ivory Gulls have a more recent divergence time from the most recent common ancestor, and thus have not had time to develop high genetic diversity. However, Ivory Gulls last shared a common ancestor with Sabine's Gulls and kittiwakes around 2.0 MYA, in contrast to other gull species, which separated from each other during the last million years (Crochet, Bonhomme and Lebreton 2000). Ivory Gull genotypes form a star-like phylogeny around a single common haplotype, with only one-three differences, and so may not have had separate allopatric populations during the Pleistocene glaciations.

Many arctic species are characterized by weak structure and this is thought to be due to the recent establishment of colonies since the last glacial period and/or to long-distance dispersal events (Birt-Friesen *et al.* 1992; Patirana 2000; Moum and Arnason 2001; Burg *et al.* 2003). Genetic variability is also considered to be lower in birds that scavenge, as a result of the small effective population size due to a higher position in the food chain (Barrowclough and Gutierrez 1990) even in species that maintain a substantial home-range (e.g. Andean Condors Hendrickson *et al.* 2003)

The estimated long-term effective population size for Ivory Gulls is approximately 2500-5000 individuals, which is close to the suggested current census population. Given that census sizes are always much larger than effective population sizes this is an alarmingly low census size and emphasizes the species' Endangered status. There are no data available to determine the percentage of breeding individuals relative to the overall census estimate of population size. Given that Ivory Gulls have low breeding success due to heavy predation (McDonald 1976) and harsh climate (Volkov and de Korte 1996) the average success of any individual is likely quite low. The estimated population size for Ivory Gulls is similar to that estimated for other arctic bird species such as Red-legged Kittiwakes (Patirana, Hatch and Friesen 2002), Pink-footed Geese (Ruokonen, Aarvak and Madsen 2005) and Common Murres (Moum and Arnason 2001) but lower than Razorbills (Moum and Arnason 2001). Unlike many bird species that breed in the Arctic, Ivory Gulls are very well-adapted to freezing weather and prefer abundant sea ice and so may have more easily maintained their population during ice ages. Predictions that the extent and thickness of sea ice is in rapid decline (Johannessen *et al.* 1999) and may disappear

entirely do not bode well for the Ivory Gull, which are seldom seen in open water (Mehlum 1990).

Analysis of the temporal genetic diversity showed no conclusive evidence that Ivory Gull genetic diversity has declined in the post-1950 samples relative to the pre-1950 samples. Due to the small number of samples within the past 10 years (only four and all from Newfoundland), I was unable to accurately test if the recent decline in Canada has effected genetic diversity values and what the current level of genetic diversity is. Since there is no conclusive information about why the Ivory Gulls are declining and whether this is a global phenomenon, it will be difficult to preserve the population and its already low genetic diversity.

There are still questions that need to be answered about the population structure of Ivory Gulls. In the absence of Russian samples, I was unable to test the hypothesis that the Alaskan samples comprise mainly Russian individuals. As well, not all individuals had each gene region sequenced. The main area where this was an issue was in the ND4-3 region where only one Alaskan sample was sequenced. This prevented this region from being added to the overall phylogenetic tree. The Alaskan samples were also underrepresented in the ND4-1 region but since there were not any significant parsimony informative sites it was possible to presume the common haplotype. Overall, this study provided a lot of essential information about the population structure and genetic diversity of Ivory Gulls but there is still more work to be done before this enigmatic species is fully understood.

References

Anker-Nilssen, T., Bakken, V., Strøm, H., Golovkin, A., Bianki, V. and Tatarinkova, I. 2000. The Status of Marine Birds Breeding in the Barents Sea Region. Norsk Polarinstitutt Rapp. Ser. No. 113.

Ardern, S.L. and Lambert, D.M. 1997. Is the black robin in genetic peril? Molecular Ecology 6: 21-28.

Avise, J.C., Nelson, W., Bowen, B. and Walker, D. 2000. Phylogeography of colonial nesting seabirds, with special reference to global matrilineal patterns in the Sooty Tern (*Sterna fuscata*). Molecular Ecology 9: 1783-1792.

Avise, J.C. and Walker, D. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London B265: 457-463.

Baker A.J. and Marshall, H.D. 1997. Mitochondrial control region sequences as tools for understanding evolution. *In* Avian molecular evolution and systematics. D.P. Mindell ed. Academic Press, San Diego. Pp 51-82.

Ballard, J.W. and Whitlock, M.C. 2004. The incomplete natural history of mitochondria. Molecular Ecology 13: 729-744.

Barrowclough, G.F. and Gutierrez, R.J. 1990. Genetic variation and differentiation in the Spotted Owl (*Strix occidentalis*). Auk 107: 737-744.

Bateson, P.P.G. and Plowright, R.C. 1959. The breeding biology of the Ivory Gull in Spitsbergen. British Birds 52: 105-114

Birkenmajer, K. 1969. Observations on Ivory Gull *Pagophila eburnea* (Phipps) in south Vestspitsbergen. Acta Ornitologica 11: 461-476.

Birt-Friesen, V.L., Montevecchi, W., Gaston, A. and Davidson, W. 1992. Genetic structure of Thick-billed Murre (*Uria lomvia*) populations examined using direct sequence analysis of amplified DNA. Evolution 46: 267-272.

Braune, B.M., Mallory, M.L. and Gilchrist, H.G. 2006. Elevated mercury levels in a declining population of ivory gulls in the Canadian Arctic. Marine Pollution Bulletin 52: 969-987.

Buckman, A. H., Norstrom, R., Hobson, K., Karnovsky, N., Duffe, J. and Fisk, A. 2004. Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. Environmental Pollution 128: 327-338.

Burg, T.M., Lomax, J., Almond, R., de L. Brooke, M. and Amos, W. 2003. Unravelling dispersal patterns in an expanding population of a highly mobile seabird, the Northern

Fulmar (*Fulmarus glacialis*). Proceedings of the Royal Society of London B 270: 979-984.

Chesser, R.K. 1991a. Genetic diversity and female philopatry. Genetics 127: 437-447.

Chesser, R.K. 1991b. Influence of gene flow and breeding tactics on gene diversity within populations. Genetics 129: 573-583.

Cooper, A. 1994. DNA from museum specimens. In: Herrmann B., Herrmann, S. (eds) Ancient DNA: recovery and analysis of genetic material from paleontological, archaeological, museum, medical and forensic specimens. Springer, New York.

COSEWIC. 2006. COSEWIC assessment and update status report on the Ivory Gull *Pagophila eburnea* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 42pp.

Cramp, S. and K.E.L. Simmons. 1983. Birds of the Western Palearctic. v.3. New York: Oxford University Press.

Crochet, P-A., Bonhomme, F. and Lebreton, J.D. 2000. Molecular phylogeny and plumage evolution in gulls (*Larini*). Journal of Evolutionary Biology 13: 47-57.

Crochet, P-A. and Desmarais, E. 2000. Slow rate of evolution in the mitochondrial control region of gulls (*Aves : Laridae*). Molecular Biology and Evolution 17: 1797-1806.

Dalgety, C.T. 1932. The Ivory Gull in Spitsbergen. British Birds 26:2-7.

de Korte, D.E.and Volkov, A.E. 1993. Large colony of Ivory Gulls *Pagophila eburnea* at Domashniy Island, Severnaya Zemlya. Sula 7: 107-110.

Dynesius, M. and Jansson, R. 2000. Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. Proceedings of National Academy of Sciences 97: 9115-9120.

Excoffier, L., Laval, G. and Schneider, S. 2005 Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Fisk, A., Hobson, K. and Norstrom, R. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. Environmental Science & Technology 35: 732-738.

Frankham, R. 1998. Inbreeding and extinction: Island populations. Conservation Biology 12: 665-675.

Frankham, R. and Ralls, K. 1998. Inbreeding leads to extinction. Nature 392: 441-442.

Fu, Y.X. 1997. Statistical neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915-925.

Genovart, M., Oro, D. and Bonhomme, F. 2003. Genetic and morphological differentiation between the two largest breeding colonies of Audouin's Gull *Larus audouinii*. Ibis 145: 448-456.

Gilchrist, H.G. and Mallory, M. 2005. Declines in abundance and distribution of the ivory gull (*Pagophila eburnea*) in Arctic Canada. Biological Conservation 121: 303-309.

Glenn, T.C., Stephan, W. and Braun, M.J. 1999. Effects of a Population Bottleneck on Whooping Crane Mitochondrial DNA Variation. Conservation Biology 13: 1097-1107.

Haig, S.M. 1998. Molecular contributions to conservation. Ecology 79: 413-425.

Haney, J.C. and MacDonald, S.D. 1995. Ivory Gull (*Pagophila eburnea*). *In* The Birds of North America, No. 175 (Poole, A. and Gill, F., eds). The Birds of North America, Inc., Philadelphia.

Haney, J.C. 1993. Rare, local, little known and declining North American breeders. A closer look: Ivory Gull. Birding 24: 330-338.

Hendrickson, S.L., Bleiweiss, R., Matheus, J., de Matheus, L., Jacome, N. and Pavez, E. 2003. Low genetic variability in the geographically widespread Andean Condor. Condor 105: 1-12.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnaean Society 58: 247-276.

Hjort, C. 1976. An observation of Ivory Gull, *Pagophila eburnea*, migration along the East Greenland current. Dansk ornithologisk Forenings Tidsskrift. 70: 72-73.

Ilyichev, V.D. and Flint, V.E. 1998. Ivory Gull *In* Birds of the USSR: Larids. Nauka, Moscow. 51-57.

Kidd, M.G. and Friesen, V.L. 1998. Analysis of mechanisms of micro-evolutionary change in *Cepphus* guillemots using patterns of control region variation. Evolution 52: 1158-1168.

Krajick, K. 2003. In search of the Ivory Gull. Science 301: 1840-1841.

Johannessen, O.M., Shalina, E.V. and Miles, M.W. 1999. Satellite evidence for an Arctic sea ice cover in transformation. Science 286: 1937-1939.

Johnson, J.A. and Dunn, P.O. 2006. Low genetic variation in the Heath Hen prior to extinction and implications for the conservation of Prairie-Chicken populations. Conservation Genetics 7: 37-48.

Lacy, R.C. 1987. Loss of genetic diversity from managed populations: interacting effects of drift, mutation, immigration, selection and population subdivision. Conservation Biology 1: 143-158.

Liebers, D. and Helbig, A.J. 2002. Phylogeography and colonization history of Lesser Black-backed Gulls (*Larus fuscus*) as revealed by mtDNA sequences. Journal of Evolutionary Biology 15: 1021-1033.

Liebers, D., Helbig, A.J. and De Kniff, P. 2001. Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* group (Aves: Charadriiformes). Molecular Ecology 10: 2447-2462.

MacDonald, S.D. 1976. Phantoms of the polar pack-ice. Audubon. 78: 2-19.

Marshall, H.D. and Baker, A.J. 1997. Structural conservation and variation in the mitochondrial control region of Fringilline finches (*Fringilla* spp.) and the Greenfinch (*Carduelis chloris*). Molecular Biology and Evolution 14: 173-184.

Martin, P.R. and McKay, J.K. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. Evolution 58: 938-945.

Mehlum, F. 1990. Seabird distribution in the northern Barents Sea marginal ice-zone during late summer. Polar Research 8: 61-65.

Moum, T. and Arnason, E. 2001. Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. Molecular Ecology. 10: 2463-2478.

Murata, K., Satou, M., Matsushima, K., Satake, S. and Yamamoto, Y. 2004. Retrospective estimation of genetic diversity of an extinct Oriental White Stork *Ciconia boyciana* population in Japan using mounted specimens and implications for reintroduction programs. Conservation Genetics 5: 553-560.

Orr, C.D. and Parsons, J.L. 1982. Ivory Gulls *Pagophila eburnea* and ice-edges in Davis Strait and the Labrador Sea. Canadian Field-Naturalist 96: 323-328.

Patirana, A., Hatch, S.A. and Friesen, V.L. 2002. Population differentiation in the Red-legged Kittiwake (*Rissa brevirostris*) as revealed by mitochondrial DNA. Conservation Genetics 3: 335-340.

Patirana, A. 2000. Global population genetics and evolutionary history of Black-legged Kittiwakes (*Rissa tridactyla*) as revealed by mitochondrial and nuclear DNA. Unpublished M.Sc. thesis. Queen's University.

Pearce, J.M. 2006. Minding the gap: frequency of indels in mtDNA control region sequence data and influence on population genetic analyses. Molecular Ecology 15: 333-341.

Pearce, J.M., Talbot, S., Pierson, B., Petersen, M., Scribner, K., Dickson, D.L. and Mosbech, A. 2004. Lack of spatial genetic structure among nesting and wintering King Eiders. Condor 106: 229-240.

Ponomarev, A., Tatarinova, T., Bubyakina, V., Smagulova, F., Kashentseva, T. and Morozov, I. 2004. Variation of mitochondrial DNA D-loop sequences in the endangered Siberian crane *Grus leucogeranus Pallas*. Conservation Genetics 5: 847-851.

Pons, J-M., Hassanin, A. and Crochet, P-A. 2005. Phylogenetic relationships within the *Laridae* (Charadriiformes: *Aves*) inferred from mitochondrial markers. Molecular Phylogenetics and Evolution 37: 686-699.

Porsild, A.E. 1943. Birds of the Mackenizie Delta. Canadian Field-Naturalist 57: 19-35.

Randi, E. and Lucchini, V. 1998. Organization and evolution of the mitochondrial DNA control region in the avian genus *Alectoris*. Journal of Molecular Evolution 47: 449-462.

Reed, D.H. and Frankham, F. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17: 230-237.

Reed, D.H., Briscoe, D.A. and Frankham, F. 2002. Inbreeding and extinction: The effect of environmental stress and lineage. Conservation Genetics 3: 301-307.

Renaud, W.E. and McLaren, P.L. 1982. Ivory Gull (*Pagophila eburnea*) distribution in late summer and autumn in eastern Lancaster Sound and western Baffin Bay. Arctic 35: 141-148.

Ross, J.D., Arndt, A., Smith, R., Johnson, J. and Bouzat, J. 2006. Re-examination of the historical range of the Greater Prairie Chicken using provenance data and DNA analysis of museum collections. Conservation Genetics 7: 735-751.

Rozas, J. and Rozas, R. 1999. DNASP, Version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15: 174-175.

Ruokonen, M., Aarvak, T. and Madsen, J. 2005. Colonization history of the high-arctic Pink-footed Goose *Anser brachyrhynchus*. Molecular Ecology 14: 171-178.

Salomonsen, F. 1979. Fra Zoologisk Museum XXV. Trettende forelobige liste over genfundne gronlandske ringfugle. Dansk Ornithologisk Forening Tidsskrift. 73: 191-206.

Salomonsen, F. 1967. Fuglene pa Groland. Rhodos, Copenhagen.

Selander, R.K. and Kaufman, D.W. 1973. Genetic variability and strategies of adaptation in animals. Proceedings of the National Academy of Sciences 70: 1875-1877.

Shephard, J.M., Hughes, J., Catterall, C. and Olsen, P. 2005. Conservation status of the White-Bellied Sea Eagle *Haliaeetus leucogaster* in Australia determined using mtDNA control region sequence data. Conservation Genetics 6: 413-429.

Slack, K.E., Delsuc, F., Mclenachan, P., Arnason, U. and Penny, D. 2007. Resolving the root of the avian mitogenomic tree by breaking up long branches. Molecular Phylogenetics and Evolution 42: 1-13.

Spielman, D., Brook, B.W. and Frankham, R. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences USA 101: 15261-15264.

Stenhouse, I.J., Robertson, G.J. and Gilchrist, H.G. 2004. Recoveries and survival rates of ivory gulls (*Pagophila eburnea*) banded in Nunavut, Canada, 1971-1999. Waterbirds 27: 486-492.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585-595.

Tiedemann, R., Paulus, K., Scheer, M., Von Kistowski, K., Skirnisson, K., Bloch, D. and Dam, M. 2004. Mitochondrial DNA and microsatellite variation in the Eider Duck (*Somateria mollissima*) indicate stepwise postglacial colonization of Europe and limited current long-distance dispersal. Molecular Ecology 13: 1491-1494.

Tomkovich, P.S. 1990. On the post-fledging movements of Ivory Gulls (*Pagophila eburnea*). Modern Ornithology 150-151.

Tuck, L.M. 1971. The occurrence of Greenland and European birds in Newfoundland. Bird-Banding 42: 184-209.

Vallianatos, M., Lougheed, S.C. and Boag, P.T. 2002. Conservation genetics of the Loggerhead Shrike (*Lanius ludovicianus*) in central and eastern North America. Conservation Genetics 3: 1-13.

Volkov, A.E. and de Korte, J. 1996. Distribution and numbers of breeding Ivory Gulls *Pagophila eburnea* in Severnaja Zemlja, Russian Arctic. Polar Research 15: 11-21.

Vuilleumier, F. 1995. A large colony of Ivory Gulls (*Pagophila eburnea*) on Victoria Island, Russia. Alauda 63: 135-148.

Wilson, A.C., Cann, R., Carr, S., George, M., Gyllensten, U., Helm-Bychowski, K., Higuchi, R., Palumbi, S., Prager, E., Sage, R. and Stoneking, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of the Linnean Society 26: 375-400.

Wright, S. 1951. The genetical structure of populations. Ann. Eugen. 15: 323-354

Zhang, B., Fang, S-G. and Xi, Y-M. Low genetic diversity in the endangered Crested Ibis *Nipponia nippon* and implications for conservation. Bird Conservation International 14: 183-190.

Zubakin, V.A. 1984. Ivory Gull *In Red Data book of the USSR*, 2nd ed., A.M. Borodin *et al.* eds, Leshnaya Promishlennost, Moscow.

Table 2.2.1 Ivory Gull primer sequence pairs with annealing information

Primer Name	Sequence	Annealing Temperature and Time
GullCR1-F	CCT ACA CCC CTA GCC CAT CTT GCT CTT TTG	50 °C for 0:45
GullCR1-R	CCA GTT GTT TGG CAA AGT GCA TCA GTG AGG	
GullCR#1F	TCA GCA ACC CGG TGT AGG AAA GAT CCT ACG	52 °C for 0:35
GullCR#1R	ATC ACG GTT AAT CTT TCA GTT AAA ACT TCC	
Gull12S2F	AAA GCA TGG CAC TGA AGA TGC CAA GAT GGC	52.4 °C for 0:35
Gull12S1R	GCA TCG AGA TTT AGG GCT AGG CAT AGT GG	
GullND4-1F	CAC CTC CAC AAC CTA AAC CTA CTA CAA TGC	50 °C for 1:00
GullND4-1R	GGG TGA TGA GAA TTA GGG TGG GGA TTA AGG	
GullND4LF	ATT TCG GCT CAA CAA ACC ATA GTC TAA CCC	50 °C for 1:00
GullND4LR	GCG ATT AAC AGG CTG TAT ATG GTG GTG TTT	

Table 2.3.1 Polymorphic sites in the Control Region of the Ivory Gull

	Base Pair				
Haplotype	127	178	232	244	249
H1	T	A	G	C	T
H2	T	A	**A**	C	T
H3	**:**	A	G	C	T
H4	T	A	**A**	**T**	T
H5	T	A	G	C	**C**
H6	T	**C**	G	C	T
H7	T	A	G	**T**	T

Table 2.3.2 Haplotype distribution of Ivory Gull Control Region samples

Haplotype Distribution	H1	H2	H3	H4	H5	H6	H7	Total
Canada Breeding	21	3	1	0	1	0	0	**26**
Greenland Breeding	14	1	0	0	0	0	0	**15**
Norway Breeding	11	4	1	1	0	0	0	**17**
Canada Non-Breeding	19	6	1	1	0	0	0	**27**
Greenland Non-Breeding	8	2	0	0	0	1	0	**11**
Alaska Non-Breeding	17	3	9	0	0	0	1	**29**
Total	**90**	**19**	**12**	**2**	**1**	**1**	**1**	**126**

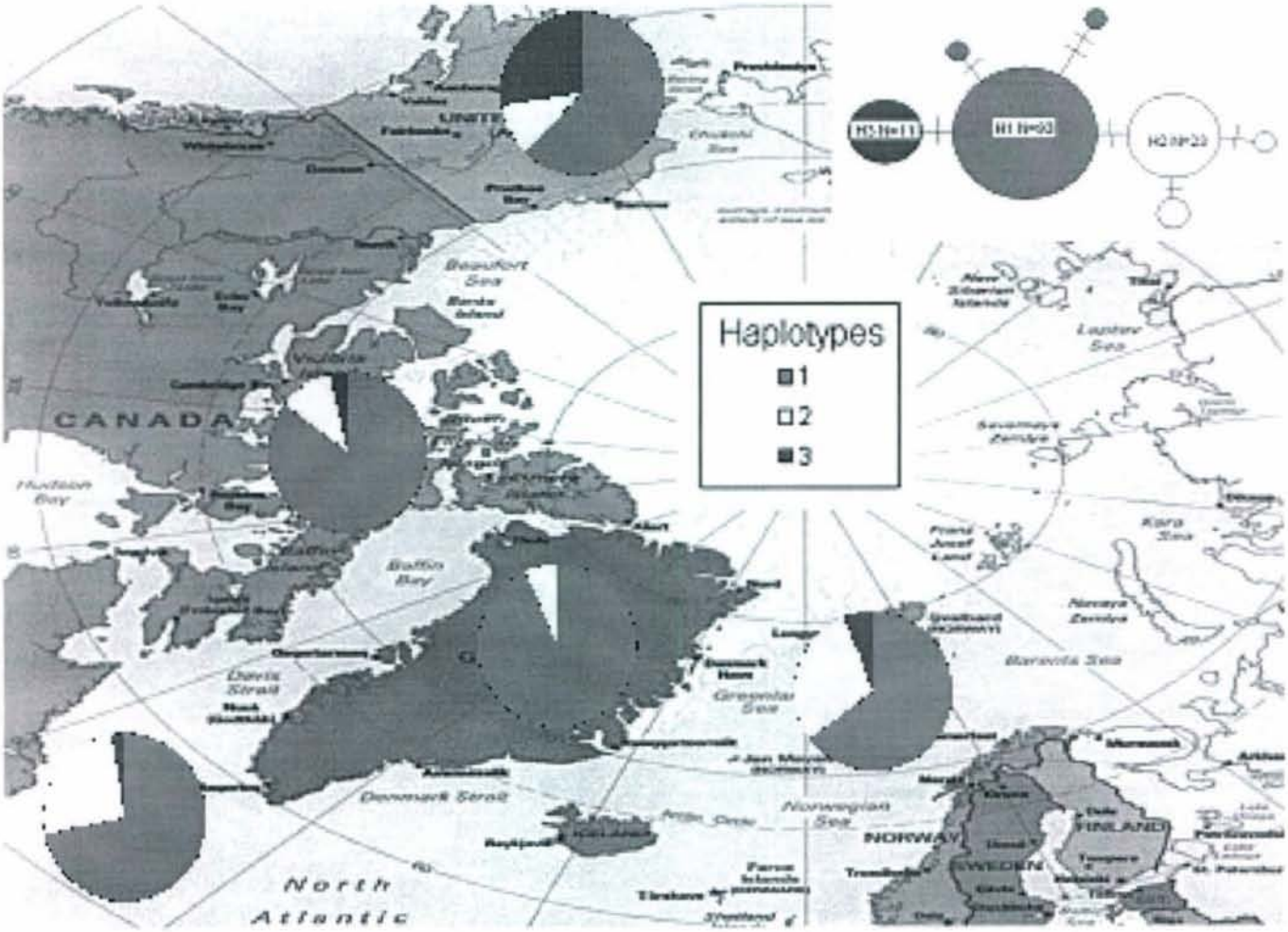

Figure 2.3.1 Locational distribution of control region haplotype clades

The distribution of haplotype groups (H1 in red, H2 in yellow and H3 in blue) over each breeding or non-breeding group.

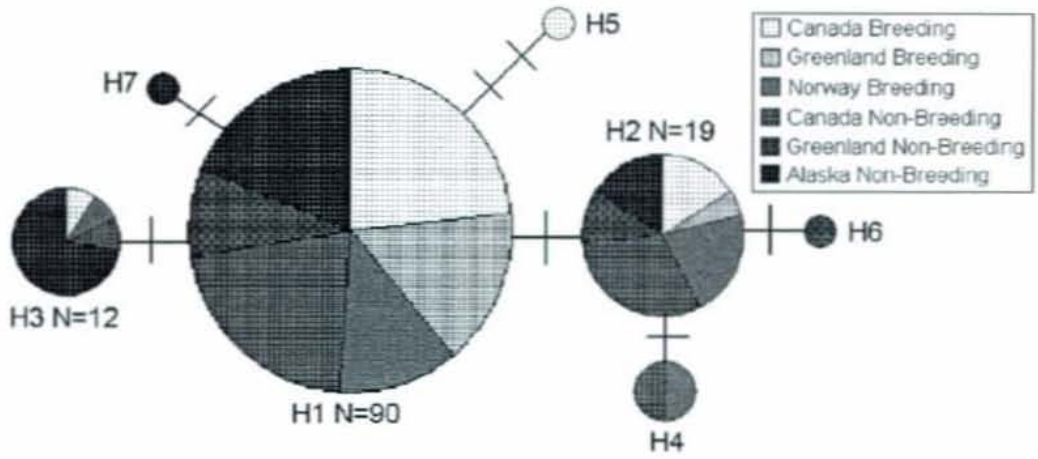

Figure 2.3.2 Minimum Spanning Network of Ivory Gull Control Region sequence data

Table 2.3.3 Genetic diversity using Ivory Gull Control Region sequence by sampling location

Location	Individuals	Haplotypes	Hd	π	k
Canada Breeding	26	4	0.345	0.00168	0.443
Greenland Breeding	15	2	0.133	0.000510	0.133
Norway Breeding	17	4	0.551	0.00256	0.676
Canada Non-Breeding	27	4	0.470	0.00207	0.547
Greenland Non-Breeding	11	3	0.472	0.00234	0.618
Alaska Non-Breeding	29	4	0.589	0.00256	0.675

Table 2.3.4 Analysis of molecular variance (AMOVA) of Ivory Gull Control Region sequence data

AMOVA Model	Groups	Variance Component	Variance Fraction	Significance
#1- One group; all 6 populations	(1)CanB,GrnB, NorB, CanNB, GrnNB,AlaNB	AP: 0.0118 WP: 0.265	AP: 0.0427 WP: 0.957	F_{ST}: 0.0284
#2- Two breeding groups	(1) CanB,GrnB (2) NorB	AG: 0.0194 AP: -0.00795 WP: 0.216	AG: 0.0851 AP: -0.0350 WP: 0.950	F_{CT}: 0.329 F_{SC}: 1.00 F_{ST}: 0.205
#3- Two breeding groups with Alaska representing Russia	(1) CanB, GrnB (2) NorB, AlaNB	AG: 0.00722 AP: 0.0112 WP: 0.257	AG: 0.0262 AP: 0.0405 WP: 0.933	F_{CT}: 0.310 F_{SC}: 0.0870 F_{ST}: 0.0186
#4- Three breeding groups with Alaska representing Russia	(1) CanB, GrnB (2) NorB (3) AlaNB	AG: 0.0305 AP: -0.0101 WP: 0.257	AG: 0.110 AP: -0.0364 WP: 0.927	F_{CT}: 0.172 F_{SC}: 1.00 F_{ST}: 0.0176
#5- Two non-breeding groups	(1) CanNB, GrnNB (2) AlaNB	AG: 0.0461 AP: -0.0141 WP: 0.307	AG: 0.136 AP: -0.0414 WP: 0.906	F_{CT}: 0.337 F_{SC}: 0.889 F_{ST}: 0.0264

Table 2.3.5 Pair-wise Φ_{ST} values for Ivory Gull Control Region sequence data with corresponding P below

	Canada Breeding	Greenland Breeding	Norway Breeding	Canada Non-Breeding	Greenland Non-Breeding	Alaska Non-Breeding
Canada Breeding	-	-0.0331	0.0234	0.0126	0.0111	0.0661
Greenland Breeding	0.999 ns	-	0.0706	0.0451	0.0676	0.0978
Norway Breeding	0.218 ns	0.179 ns	-	-0.0464	-0.0537	0.0722
Canada Non-Breeding	0.277 ns	0.217 ns	0.999 ns	-	-0.0463	0.0883
Greenland Non-Breeding	0.278 ns	0.233 ns	0.927 ns	0.894 ns	-	0.0994
Alaska Non-Breeding	0.0430 *	0.0430 *	0.0640 ns	0.0210 *	0.0490 *	-

* = $P < 0.05$ ns = $p > 0.05$

Table 2.3.6 Neutrality tests of Ivory Gull Control Region sequence data

Sample Location	Tajima's D	P	Fu's F	P
Canada Breeding	-1.54	0.0560 ns	-1.60	0.0450 *
Greenland Breeding	-1.16	0.133 ns	-0.649	0.0920 ns
Norway Breeding	-0.673	0.273 ns	-1.06	0.105 ns
Canada Non-Breeding	-0.721	0.254 ns	-1.09	0.185 ns
Greenland Non-Breeding	-0.290	0.412 ns	-0.314	0.299 ns
Alaska Non-Breeding	-0.277	0.410 ns	-0.556	0.293 ns

Table 2.3.7 Genetic diversity values for different mitochondrial markers in Ivory Gulls

Marker	Base Pairs	Samples	Nucleotide Diversity	Haplotype Diversity
CR	264	126	0.00207	0.451
12S rRNA	620	105	0.000440	0.254
ND4-1	427	97	0.000570	0.101
ND4-3	640	73	0.000700	0.405
ND4L	439	112	0.000280	0.121

Table 2.3.8 Polymorphic sites in the Ivory Gull combined sequence

	Region and Base Pair																				
	CR					12S				ND4-1							ND4L				
	127	178	232	244	249	295	441	465	482	101	226	236	243	258	329	351	65	161	331	374	425
H1	T	A	G	C	T	T	A	C	A	C	A	G	C	C	C	A	C	C	G	G	T
H2	T	A	**A**	C	T	**C**	A	C	A	C	A	G	C	C	C	A	C	C	G	G	T
H3	:	A	G	C	T	T	A	C	A	C	A	G	C	C	C	A	C	C	G	G	T
H4	T	A	**A**	C	T	T	A	C	A	C	A	G	C	C	C	A	C	C	G	G	T
H5	T	A	G	**T**	T	T	A	C	A	C	A	G	C	C	C	A	C	C	G	G	T
H6	T	A	G	C	T	T	A	C	A	C	A	G	C	C	C	A	C	C	**A**	G	T
H7	T	A	G	C	**C**	T	A	C	A	C	A	G	C	C	C	A	C	C	G	G	T
H8	T	A	G	C	T	T	A	C	A	**T**	A	G	C	C	C	**G**	C	C	G	G	T
H9	T	A	G	C	T	T	A	C	A	C	A	G	C	C	C	A	C	C	G	G	**C**
H10	T	A	**A**	C	T	**C**	A	C	A	C	A	G	C	C	C	A	**T**	C	G	G	T
H11	T	A	G	C	T	T	A	C	A	C	A	G	**G**	C	**T**	A	C	C	G	G	T
H12	T	A	G	C	T	**C**	A	C	A	C	A	G	C	C	C	A	C	C	G	G	T
H13	T	A	**A**	C	T	**C**	A	C	A	C	A	G	C	C	C	A	C	C	G	G	**C**
H14	T	**C**	**A**	C	T	T	A	C	:	C	A	G	C	C	C	A	C	C	G	G	T
H15	T	A	G	C	T	T	A	C	A	C	**G**	**A**	C	**T**	C	A	C	C	G	G	T
H16	T	A	**A**	C	T	T	A	C	A	C	A	G	C	C	C	A	C	**T**	G	G	T
H17	T	A	G	C	T	T	**G**	C	A	C	A	G	C	C	C	A	C	C	G	G	T
H18	T	A	G	**T**	T	T	A	**T**	A	C	A	G	C	C	C	A	C	C	G	G	T
H19	T	A	G	C	T	T	A	**T**	A	C	A	G	C	C	C	A	C	C	G	G	T
H20	T	A	G	C	T	T	A	C	A	C	A	G	C	C	C	A	C	C	G	**A**	T

Table 2.3.9 Haplotype distribution of Ivory Gull combined sequence data

	Canada Breeding	Greenland Breeding	Norway Breeding	Canada Non-Breeding	Greenland Non-Breeding	Alaska Non-Breeding	Total
H1	21	13	9	17	6	15	**81**
H2	3		1	5		0-2	**9-11**
H3	1		1	1		9	**12**
H4			2	1	1	1-3	**5-7**
H5				1			**1**
H6				1			**1**
H7	1						**1**
H8	1						**1**
H9	1						**1**
H10			1				**1**
H11			1				**1**
H12			1				**1**
H13					1		**1**
H14					1		**1**
H15		1			1		**2**
H16		1					**1**
H17					1		**1**
H18						1	**1**
H19						1	**1**
H20						1	**1**
Total	**28**	**15**	**16**	**26**	**11**	**30**	**126**

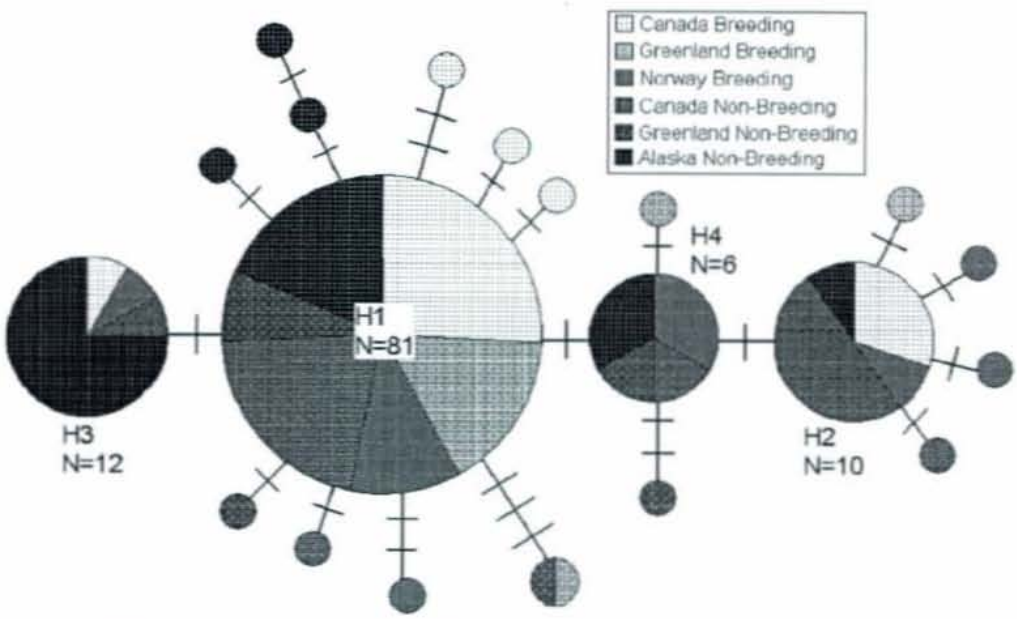

Figure 2.3.3 Minimum Spanning Network of Ivory Gull combined sequence data

Table 2.3.10 Locational genetic diversity values for Ivory Gull combined sequence data

Location	N	H	Hd	π	k
Canada Breeding	26	6	0.465	0.000510	0.886
Greenland Breeding	15	3	0.257	0.000380	0.667
Norway Breeding	17	8	0.728	0.000900	1.54
Canada Non-Breeding	27	7	0.547	0.000600	1.05
Greenland Non-Breeding	11	5	0.618	0.000870	1.53
Alaska Non-Breeding	29	7	0.670	0.000540	0.946

Table 2.3.11 Analysis of molecular variance (AMOVA) of Ivory Gull combined sequence data

AMOVA Model	Groups	Variance Component	Variance Fraction	Significance
#1- One group; all 6 populations	CanB, GrnB, NorB, CanNB, GrnNB, AlaNB	AP: 0.0171-0.0225 WP: 0.519-0.539	AP: 0.0309-0.0413 WP: 0.959-0.969	F_{ST}: 0.0108-0.0362
#2- Two breeding groups (West vs. East)	(1) CanB, GrnB (2) NorB	AG: 0.0283-0.0361 AP: -0.00624- -0.00568 WP: 0.511-0.522	AG: 0.0531-0.0655 AP: -0.0113- -0.0107 WP: 0.946-0.958	F_{CT}: 0.329-0.335 F_{SC}: 0.369-0.377 F_{ST}: 0.0831-0.105
#3- Two breeding groups with Alaska representing Russia	(1) CanB, GrnB (2) NorB, AlaNB	AG: -0.00407-0.00273 AP: 0.0200-0.0323 WP: 0.486-0.516	AG: -0.00780-0.00510 AP: 0.0375-0.0619 WP: 0.946-0.957	F_{CT}: 0.345-0.665 F_{SC}: 0.00980-0.0567 F_{ST}: 0.00390-0.0147
#4- Three breeding groups with Alaska representing Russia	(1) CanB, GrnB (2) NorB (3) AlaNB	AG: 0.0319-0.0399 AP: -0.00595- -0.00440 WP: 0.486-0.516	AG: 0.0596-0.0755 AP: -0.0109- -0.00850 WP: 0.934-0.951	F_{CT}: 0.162-0.175 F_{SC}: 0.358-0.388 F_{ST}: 0.00490-0.0176
#5- Two non-breeding groups	(1) CanNB, GrnNB (2) AlaNB	AG: 0.0418-0.0515 AP: -0.00335- -0.00148 WP: 0.525-0.554	AG: 0.0705-0.0895 AP: -0.00560- -0.00260 WP: 0.913-0.935	F_{CT}: 0.333-0.353 F_{SC}: 0.385-0.404 F_{ST}: 0.0127-0.0352

Table 2.3.12 Population Pair-wise Φ_{ST} values for Ivory Gull combined sequence data with corresponding P below

	Canada Breeding	Greenland Breeding	Norway Breeding	Canada Non-Breeding	Greenland Non-Breeding	Alaska Non-Breeding
Canada Breeding	-	-0.000120	0.0193 or 0.0264	0.00709	-0.00570	0.0389 or 0.0459 or 0.0559
Greenland Breeding	0.355-0.379	-	0.0562 or 0.0707	0.0633	-0.00719	0.0646 or 0.0662 or 0.0709
Norway Breeding	0.143-0.196	0.0330-0.0580	-	-0.0175 or -0.0198	-0.0185 or -0.114	0.0598 or 0.0817 or 0.0969
Canada Non-Breeding	0.255-0.280	0.0610-0.0770	0.618-0.661	-	-0.00989	0.0692 or 0.0824 or 0.0984
Greenland Non-Breeding	0.381-0.403	0.599-0.613	0.508-0.617	0.392-0.425	-	0.0667 or 0.0738 or 0.0837
Alaska Non-Breeding	0.0180-0.0720	0.0250-0.0380	0.00900-0.0400	0.00600-0.0310	0.0270-0.0520	-

Table 2.3.13 Neutrality tests using Ivory Gull combined sequence data

Sample Location	Tajima's D	P	Fu's F	P
Canada Breeding	-1.82	0.0110-0.0200 *	-2.22	0.0360-0.0560
Greenland Breeding	-1.91	0.00500-0.0150 *	0.106	0.394-0.440 ns
Norway Breeding	-0.756 or -0.878	0.193-0.266 ns	-3.47 or -3.65	0.00200-0.0130 *
Canada Non-Breeding	-0.946	0.173-0.233 ns	-2.74	0.0180-0.0330 *
Greenland Non-Breeding	-1.46	0.0640-0.0820 ns	-1.03	0.167-0.186 ns
Alaska Non-Breeding	-0.857 or -0.976 or -1.10	0.134-0.218 ns	-2.10 or -2.77 or -2.01	0.0150-0.0600

Table 2.4.1 Genetic diversity values for relevant avian species

Species	Comment	Haplotype Diversity	Nucleotide Diversity	Reference
Ross's Gull (*Rhodostethia rosea*)	-	0.00430	0.769	Chapter 3 of this thesis
Ivory Gull (*Pagophila eburnea*)	-	0.00207	0.451	Chapter 2 of this thesis
Whooping Crane (*Grus americana*)	Endangered species	0.0045	0.0044	Glenn *et al.* 1999
Heath Hen (*Tympanuchus cupido cupido*)	Extinct species	0.363	0.009	Johnson and Dunn 2006
Crested Ibis (*Nipponia nippon*)	Endangered species	0.386	0.00069	Zhang *et al.* 2004
Pink-footed Goose (*Anser brachyrhynchus*)	Arctic species	0.51	0.003	Ruokonen *et al.* 2005
Andean Condor (*Vultur gryphus*)	Scavenging species	0.59	0.0020	Hendrickson *et al.* 2003
Three-toed Woodpecker (*Picoides tridactylus*)	Arctic species	0.63	0.001	Zink *et al.* 2002
Rock Ptarmigan (*Lagopus mutus*)	Arctic species	0.70	0.002	Holder *et al.* 2000
Common Murre (*Uria aalge*)	Arctic species	0.72	0.005	Moum and Arnason 2001
Siberian Crane (*Grus leucogeranus*)	Endangered Arctic species	0.9	0.0060	Ponomarev *et al.* 2004
Red-legged Kittiwake (*Rissa brevirostris*)	Close relative	0.91	0.015	Patirana *et al.* 2002
Razorbill (*Alca torda*)	Arctic species	0.92	0.0126	Moum and Arnason 2001
Common Eider (*Somateria mollissima*)	Arctic species	0.92	0.0175	Tiedemann *et al.* 2004

Chapter 3: Genetic Diversity and Differentiation of Ross's Gull (*Rhodostethia rosea*)

Introduction

Ross's Gull (*Rhodostethia rosea*) is the rarest breeding gull in North America as the known breeding population has varied from 1-5 pairs however, the Canadian population is thought to have always been small, despite large areas of potential habitat (Alvo *et al.* 1996). Only four breeding locations have been confirmed: Cheyne Islands, Nunavut (MacDonald 1978); Churchill, Manitoba (Chartier and Cooke 1980); Prince Charles Island, Nunavut (Bechet *et al.* 2000); and an unnamed island in Nunavut (Mallory *et al.* 2006). The breeding success of Ross's Gulls in Canada has been fairly low due to bad weather and predation by Arctic foxes (*Alopex lagopus*), Glaucous Gulls (*Larus hyperboreus*) and weasels (*Mustela frenata*) (Densley 1999). Disturbance has become an increasing problem, especially in Churchill, and has resulted in several cases of unsuccessful nesting attempts (Alvo *et al.* 1996). The Canadian population of Ross's Gull is classified as Threatened by COSEWIC (Committee on the Status of Endangered Wildlife in Canada) due to its small population and low productivity (Alvo *et al.* 1996).

Ross's Gull is a circumpolar species breeding in the Subarctic, Low Arctic and High Arctic areas although roughly 95% of its breeding population is found in northeastern Siberia, between the Chukotka and Taymyr Peninsulas (Zubakin *et al.* 1990). In 1978, the Russian population was estimated at approximately 10,000 sexually mature

birds (Alvo *et al.* 1996). The world population calculated by Bannikow and Flint (1978) of 10,000 is now thought to have been underestimated as recent censuses of Siberian breeding grounds suggest that the world population may be as many as 50,000 individuals (Alvo *et al.* 1996). However, according to a recent survey of northern Yakutia, Russia, Ross's Gull is more widespread than has been previously assumed (CAFF 2004). The Conservation of Arctic Flora and Fauna (CAFF) group (2004) has suggested that the current population estimate of 100,000 birds might be low, which would make the global population much larger than any previous estimate.

In mid-September, after the summer breeding season, Ross's Gulls move from the Russian Chukchi Sea to the Point Barrow, Alaska region and then into the Beaufort Sea in late September or early October (Divoky *et al.* 1988). Population estimates for Alaska (20,000 to 40,000 birds) in 1988 by Divoky *et al.* suggest that in any given year, a large proportion of the world population of Ross's Gull likely resides in the nearshore zone of the Chukchi and Beaufort Seas. There is a return movement in mid- to late-October once the Alaskan Beaufort and Chukchi Seas freeze (Divoky *et al.* 1988) to the Sea of Okhotsk (Degtyarev, Labutin and Blohin 1987). One of the major conservation concerns with Ross's Gull is the potential for the concentrated autumn population in the Chukchi and western Beaufort Seas to be devastated by a pollution event, such as an oil spill from nearby oil drilling (Alvo *et al.* 1996). The highly productive polar ice that borders the Barents and Greenland Seas serves as an important feeding and moulting area for non-breeding Ross's Gulls during the summer (Meltofte *et al.* 1981). In fact, Ross's Gulls appear to be the most common bird in the Central Arctic Ocean, north of 85°N (Hjort *et al.* 1997).

Ross's Gull is one of the least studied of the northern hemisphere seabirds and important elements of its biology not yet understood. The most comprehensive research on Ross's Gulls breeding biology remains Buturlin (1906). Phylogenetically, Ross's Gull Control Region and Cytochrome *b* sequence formed a monophyletic group with the Little Gull (*Larus minutus*; Pons, Hassanin and Crochet 2005). As a result of this genetic relationship and numerous phenotypic and behavioral similarities, Pons, Hassanin and Crochet (2005) suggested putting both species into a new genus *Hydrocoloeus* but this taxonomic change has not yet been accepted.

The extremely low Canadian population and remoteness of Ross's Gulls other breeding and wintering areas precluded using fresh tissue and so museum specimens were sequenced instead. Museum specimens have provided valuable information for avian conservation genetic studies (e.g. Greater Prairie Chicken *Tympanuchus cupido,* Bouzat *et al.* 1998; Bearded Vulture *Gypaetus barbatus,* Godoy *et al.* 2004; Red Grouse *Lagopus lagopus scoticus,* Freeland *et al.* 2006 and Ivory Gull *Pagophila eburnea,* Chapter 2). Use of museum specimens made using mitochondrial sequences more practical as they are present in much higher copy number (Ballard and Whitlock 2004) resulting in a higher probability of intact sequences (Cooper 1994). As well, the effective population size of mitochondrial DNA is lower than nuclear DNA, due to its maternal inheritance, which allows ascertainment of population bottlenecks more easily (Wilson *et al.* 1985).

Arctic species tend to have lower levels of genetic diversity (Hewitt 1996; Martin and McKay 2004) but there is considerable variation among species in the amount of genetic structure due to life-history traits such as breeding distribution, philopatry and the extent of fragmentation into refugia during ice ages (Avise and Walker 1998). For

example, this is seen in the gull family *Laridae* as northern latitude Lesser Black-backed Gulls (*Larus fuscus*) had much lower levels of genetic diversity and genetic structure than southern latitude Yellow-legged Gulls (*Larus cachinnans*) (Liebers, Helbig and De Kniff 2001; Liebers and Helbig 2002). Steller's Eider (*Polysticta stelleri*) breed mainly in Russia but a small population of conservation concern breeds in Alaska, similar to Ross's Gulls and genetic analysis of the Alaskan population with others across the species range showed a significant level of mtDNA differentiation (Pearce *et al.* 2005). Genetic diversity patterns can also provide insight into the population history of a species. For example, Razorbills (*Alca torda*) have a similar breeding distribution to Ross's Gull as only 3% of their breeding population is in North America (Nettleship and Evans 1985). When the Razorbill control region was sequenced by Moum and Arnason (2001), they found that nucleotide diversity was actually highest in the two North American colonies, suggesting that the current Razorbill population originated from a South-West Atlantic refugial population and through sequential founder events colonized the North and East Atlantic.

The distribution of Ross's Gull breeding outside of Siberia is not known but in addition to the Canadian data, there have also been at least 30 reports of Ross's Gull in Greenland, including several breeding birds (Kampp and Kristensen 1980). Taken with the information available about the Canadian breeding population, this raises several questions. Are these breeding attempts isolated intermittent incidents or do they indicate the presence of a continual breeding population? Secondly, if there is a continuous breeding population in Canada and Greenland, do they represent a separate population or are they recruited from the main Siberian population? The documented birds are few, but

the areas that Ross's Gull are likely to breed have exceedingly low human contact and so it is very possible that there are sites that remain undiscovered (Mallory *et al.* 2006). Ross's Gull is known to many Inuit in southern Baffin Island, which suggests that they may be more common that current data indicates (Mallory *et al.* 2001). There is also evidence that Ross's Gulls may move colonies each year or that colony occupation is sporadic, especially in the higher arctic areas (Mallory *et al.* 2006). However, Ross's Gull appears to have nested annually or almost annually from 1980 to 1994 in Churchill, Manitoba and Nunavut (Alvo *et al.* 1996).

The origin of Ross's Gulls breeding in Canada has long been a mystery, as it is not known whether they represent a distinctive group or are merely sporadic opportunistic breeding attempts. The extremely low breeding population in Canada, coupled with the conservation threats faced by the birds, makes it extremely important to assess the potential genetic distinctiveness of these birds. Thus, the main aim of this study was to determine whether or not the Canadian Ross's Gull samples were genetically differentiated from the main breeding population in Siberia and thus should be considered a separate management unit. The genetic diversity of the small Canadian population was also compared to the main population to provide insight on the historical population size. Genetic diversity values also helped to supply information about the population history of Ross's Gull including potential population bottlenecks and the long-term effective population size.

Materials and Methods

Samples and DNA extraction

Fourteen individuals were sequenced for the control region, eight from Canadian birds and six from Alaskan birds. Appendix 1 gives a detailed list of samples, the museums from which they were obtained, and the locations and dates of collection.

Using sterile technique, a ~1mm^2 piece of the sample was removed and DNA was extracted using QIAamp DNA Mini Kit Tissue Protocol (Qiagen Inc. and explained in depth in Chapter 2). This protocol included complete lysis of protein using proteinase K and Buffer ATL overnight, the addition of Buffer AL and 100% ethanol to precipitate the DNA and then several washes of Buffer AW1 and AW2 before dilution with dH_2O.

PCR Amplification

Gull-specific oligonucleotide primers (GullCR#1F TCAGCAACCCGGTGTAGG AAAGATCCTACG and GullCR#1R ATCACGGTTAATCTTTCAGTTAAAACTTCC) were designed for the Control Region (CR), using the Kelp Gull (*Larus dominicanus*) mtDNA genome sequence obtained by Slack *et al.* (2007) (GenBank accession NC_007006).

DNA was amplified using 15μL cocktail composed of 10μL of dH_2O, 2.5μL of 10xPCR buffer, 0.5μL of dNTPs [20mM], 0.5μL of each primer [10mM] and 0.2μL (1 U) of Hot Start Taq polymerase. The sample tubes also contained 10μL of DNA with one control tube only containing cocktail to ensure no DNA contamination. Using the Eppendorf Mastercycler, each primer-specific program started with 15 minutes at 95°C to activate the Taq polymerase activity. The PCR amplification cycle consisted of 45 seconds at 93 °C, the annealing stage which consisted of 35 seconds at 52 °C and then one

minute at 72 °C, repeated 45 times to ensure adequate DNA amplification. After the last cycle, a final amplification at 72 °C for five minutes was performed, and then the samples were held at 5 °C. The presence of amplified DNA was confirmed by running all the samples on a 2% agarose gel. Once the bands were confirmed (with no control band present) the PCR products were purified using Qiagen PCR cleanup protocol outlined in Chapter 2.

Sequencing reactions

Sequencing reactions were done in both the forward and reverse directions to ensure accuracy, using 5µL of DNA for each. The DNA was vacufuged and a 5.8µL cocktail containing 3.48µL of distilled water, 2µL of Big Dye and 0.32µL of either the forward or reverse primer per sample was added. Using the Eppendorf Mastercycler, reactions were carried out with an initial two minute separation stage at 96 °C before beginning the cycle of 0:30 at 96 °C, 0:15 at 50 °C and 4:00 at 60 °C. This cycle was repeated 45 times and then the samples were held at 5 °C.

The sequenced DNA was purified by precipitation with 75% isopropanol; centrifugation and removal of the supernatant (see Chapter 2 for details). Once purified, the samples were dried using the vacufuge and 5µL of foramide EDTA was added. The reactions were then denatured in the Eppendorf Mastercycler by heating the samples up to 95°C for two minutes and then reducing the temperature rapidly to 5°C.

Automated DNA Sequencing

Using a 96 lane ABI 377 Sequencer, the samples were sequenced on an acrylamide gel with a 48 or 64 comb containing 1μL or 0.8μL of the purified DNA sample. The acrylamide gel preparation and the protocol for operating the Sequencer are outlined in Chapter 2. The resulting gel file was exported into Sequencher.

Analysis

Sequences were aligned and Single Nucleotide Polymorphisms (SNPs) were identified using Sequencher. The number of haplotypes, overall haplotype diversity (Hd), overall nucleotide diversity (π), overall Tajima's D (Tajima 1989) and Fu's F-statistics (Fu 1997) and the average number of differences (k) were obtained using DNASP version 4.0 (Rozas and Rozas 1999). Using ARLEQUIN version 3.0 (Excoffier, Laval and Schneider 2005), nucleotide diversity, haplotype diversity, Tajima's D (Tajima 1989) and Fu's F-statistics (Fu 1997) were calculated for each separate population.

To test for population genetic structure, ARLEQUIN was used to perform analysis of molecular variance (AMOVA). AMOVA analysis allows determination of the genetic variance partitioned between different hierarchical levels based on the geographic representation of haplotypes and the pairwise distances between them. The value for F_{ST} (Wright 1951) represents the level of population structure between populations, such as Canada and Alaska (Excoffier, Smouse and Quattro 1992). Population genetic structure was further tested with ARLEQUIN using population pair-wise F_{ST} to determine the level of genetic differentiation between the Canadian and Alaskan population.

The long-term effective population size was estimated with the formula $N_e = 10^6(\pi/s)/g$, where π = the nucleotide diversity, s = rate of sequence divergence and g = the

average generation time (Wilson *et al.* 1985). The value used for the rate of sequence divergence was that obtained from the Lesser Black-backed Gull (*Larus fuscus*) control region data of 8.5% per million years (Liebers and Helbig 2002).

Results

Control Region sequence variation

I sequenced 515bp of the control region for 14 individuals, eight from Canada and six from Alaska. These sequences contained 12 polymorphic positions (2.23% of the control region sequenced), one of which was parsimony-informative (0.200%). The parsimony-informative site was a C ↔ T transition at base 294 seen in six individuals, five of which were from Canadian birds (see Table 3.3.1). The other polymorphisms were six C ↔ T transitions, four A ↔ G transitions and one C ↔ A transversion, giving an 11:1 ratio of transitions to transversions.

These polymorphic sites defined six unique haplotypes, of which four were unique to single Alaskan individuals. The singleton haplotypes are differentiated from Haplotype 2 (H2) by two to four SNPs. The remaining two haplotypes (H1 and H2) were seen in six and four individuals respectively. The Canadian samples comprised only H1 and H2 individuals whereas each of the six Alaskan individuals had a different haplotype (see Figure 3.3.1 for minimum spanning network).

The overall haplotypic diversity (Hd) was 0.769 and the nucleotide diversity (π) was 0.0043. The Alaskan haplotype diversity was twice as high and nucleotide diversity was 8 times higher than the Canadian birds (see Table 3.3.2 for values).

Geographic Structure of Control Region Sequence

There was significant population structure, albeit weak, between the Canadian and Alaskan samples. When the two groups were compared using AMOVA, 0.126 of variance was between the two groups which was significant at $p \leq 0.0400$. When further analyzed with pairwise $\Phi_{ST} = 0.126$ ($p \leq 0.0340$), supporting the AMOVA result of differentiation between the two populations.

Population History

The overall Tajima's D was -1.64 and the overall Fu's F was -2.29, neither of which were significant ($0.05 \leq p \geq 0.10$). When analyzed by population, the Canadian population had positive values for both Tajima's D and for Fu's F (see Table 3.3.4 for values), suggesting the population is in equilibrium, but these values were not significant. The Alaskan population had negative values for both tests including a significant value for Fu's F of -2.52 (p-value ≤ 0.0370), indicating this population is expanding from an earlier bottleneck (Aris-Brosou and Excoffier 1996).

The long-term effective population size was 5.1×10^3 female birds, based on the observed nucleotide diversity of 4.3×10^{-3}, generation time of 10 years and a sequence divergence rate of 8.5% per million years (from *Larus fuscus* Liebers and Helbig 2002). Most seabirds are monogamous so it can be assumed that Ross's Gulls have an approximately 1:1 sex ratio and thus an effective population size of approximately 10,000 birds. Using 20 years as the average generation time halves the estimated size.

Analyzing the populations separately for their effective population sizes using the same sequence divergence rate of 8.5% per million years (from *Larus fuscus* (Liebers and Helbig 2002)) and a generation time of 10 years results in 1180 (Canada) and 9650 (Alaska/Russia) female birds producing an effective total population of approximately 4000 Canadian and 20,000 Alaskan/Russian birds. If the generation time is assumed to be 20 years the effective population sizes are halved to 2000 Canadian and 10,000 Alaskan/Russian.

Discussion

Control Region Genetic Diversity

The overall level of genetic diversity in Ross's Gull was π =0.0043 and Hd =0.769. These values are likely underestimated due to the bias towards Canadian samples in the analysis (8 Canadian to 6 Alaskan). In contrast, in the wild, the bias is towards Alaskan/Russian birds making the true value likely closer to the values seen in that population. Gulls have been shown to have a slow rate of evolution in the control region (Crochet and Desmarais 2000) but the control region is often the most variable area of the mitochondrial genome (Baker and Marshall 1997). Red-legged Kittiwakes (*Rissa brevirostris*) had a higher value (π=0.015 and Hd=0.91-Patirana, Hatch and Friesen 2002) but Ivory Gulls (*Pagophila eburnea*) had a lower value (π=0.00207 and Hd=0.451-Chapter 2). Razorbills, an arctic species which also has a disjunct distribution with a much smaller population in North America, had higher genetic diversity (π=0.013, Hd=0.92-Moum and Arnason 2001). In contrast, the Pink-footed Goose (*Anser brachyrhynchus*), that breeds in western (Iceland and Greenland) and eastern (Svalbard)

populations, had lower values for genetic diversity (π=0.003, Hd=0.51-Ruokonen, Aarvak and Madsen 2005).

The Alaskan specimens had both a much higher haplotype (1.00 to 0.535) and nucleotide diversity (0.00820 to 0.00100) compared to the Canadian sample. This is expected, as the Russian breeding population from which the Alaskan birds likely come from may be a thousand-fold larger that the Canadian population and smaller populations carry less genetic variation than equivalent larger ones (Amos and Harwood 1998). It is also possible that some of the Canadian samples are close relatives and this is why there are only two haplotypes present. However, the Canadian samples are comprised of both Nunavut and Churchill birds, which makes it less likely that there was a direct familial relationship between the specimens sampled. Then again, the small population size of Ross's Gull in Canada increases the chance of mating between related individuals and enables the loss of rare alleles through heightened genetic drift. Inbreeding reduces population fitness and increases extinction risk, especially when the population is under environmental stress (Reed, Briscoe and Frankham 2002) making the Canadian population even more vulnerable to extirpation. The increased diversity seen in the Alaskan samples could also be a result of temporal degradation. The Alaskan specimens represent older material than the Canadian birds sampled (mean date of collection: 1934 versus 1978) and this may have caused increased diversity as an artifact of decomposition in the Alaskan samples (Sefc, Payne and Sorenson 2007) but since all samples are from museum specimens it seems unlikely that only the Alaskan samples would affected.

The distribution of genetic diversity seen in Ross's Gulls is similar to that of the Lesser White-fronted Goose (*Anser erythropus*), which have a very small (30-50 pairs)

breeding population in Fennoscandia but a much larger (~25,000) breeding population in Russia (Ruokonen *et al.* 2004). The Fennoscandia population had approximately half the haplotype and nucleotide diversity of the Russian population. The wintering population of the Lesser White-fronted Goose was also compared and Ruokonen *et al.* (2004) found that it had the highest level of genetic diversity, in agreement with the high level seen in the Alaskan wintering population of Ross's Gulls.

Geographic Structure

There is significant ($p \leq 0.040$) genetic differentiation between the Alaskan and Canadian specimens of Ross's Gulls. Since the Alaskan sample is likely representative of birds that breed in Russia, it is probable that the Russian population has a significantly different haplotype frequency structure than the Canadian population. Every individual in the Alaskan population had a different haplotype whereas the Canadian sample only had two haplotypes, both of which were also seen in Alaska. The level of differentiation seen in Ross's Gull is consistent with that seen in the Mew Gull (*Larus canus*) when birds from each side of the Bering Sea were compared. The genetic differentiation of Mew Gull had a p-value ≤ 0.02, although there is also morphological differentiation that has resulted in previous sub-species classification (*kamtschatschensis* in Russia and *brachyrhynchus* in North America) (Zink *et al.* 1995).

There are several possible explanations for the weak geographic structure. First, my sample size was small so it is possibly not representative of nature. It is conceivable that the Canadian population has existed for a long enough time at low numbers to be reduced in genetic diversity, but not long enough to have evolved unique haplotypes.

Another possibility is that the Canadian population is a recent founder population and derived from a small number of birds that originated from Russia. This scenario is supported by the presence of both H1 and H2 in the Alaskan samples. There is little information about the history of Ross's Gull in Canada but the holotype for the species was shot in June 1823 on the east coast of the Melville Peninsula in Nunavut (Blomqvist and Elander 1981). The first report of Canadian breeding was not reported until 1978 (MacDonald 1978). There are reports of breeding birds in Greenland in the 1800s (Kampp and Kristensen 1980), supporting the theory that Ross's Gull has been breeding in areas outside Russia for over one hundred years.

Despite the significant value ($p \leq 0.04$) for genetic structure of Ross's Gulls of 0.126, they have low among-group variance relative to other gull species. Lesser Black-backed Gulls had 0.21 among-group variance (Liebers and Helbig 2002) and Black-legged Kittiwakes had 0.626 variance between the Atlantic and Pacific populations (Patirana 2000). Only Ivory Gulls had a lower value (0.05, see Chapter 2), although when only Pacific colonies of Black-legged Kittiwakes were compared, they had just 0.04 among group variance (Patirana 2000).

Population History

Although the neutrality tests performed on the Canadian samples did not vary significantly from zero, there were no rare alleles, which may indicate the population may have been interrupted by a recent, substantial bottleneck (Maruyama and Fuerst 1985; Schneider and Excoffier 1999). This is consistent with the hypothesis that the current population size of Ross's Gull in Canada is very low but perhaps in the past it was higher.

The Alaskan population on the other hand, had a significantly negative value for Fu's F (-2.52, $p \leq 0.037$), although not for Tajima's D (-1.22, $p \leq 0.112$). This indicates that the Alaskan sample, and by inference the Russian population, of Ross's Gulls is expanding from a historical bottleneck. The Alaskan specimens having an excess of haplotypes support the scenario of population expansion.

Future Research

The sample size for this study is very small, especially for the Alaskan/Russian population. Analysis of additional Alaskan and Russian samples would more accurately estimate its haplotype diversity. Increasing the Alaskan/Russian samples would also help give a more accurate value for the overall genetic diversity since most Ross's Gulls are not from Canada and these samples are therefore resulting in a bias towards lower values. It would also be interesting to see if there is any population structure between the breeding areas of Siberia, as Ross's Gull nests at extremely low density over a very large area (Zubakin and Avdanin 1983). The Alaskan samples are also relatively old, mostly from the 1920s, and so current samples would be helpful to ensure that the increased diversity is not due to degradation of the sample. Due to the low population in Canada, there is little opportunity to substantially increase the sample size but additional samples from Greenland may provide more insight as they may represent the same population.

References

Alvo, R., McRae, D., Holohan, S., Divoky, G. 1996. Updated Status Report on the Ross's Gull *Rhodostethia rosea* in Canada. Committee on the Status of Endangered Wildlife in Canada.

Amos, W. and Harwood, J. 1998. Factors affecting levels of genetic diversity in natural populations. Proceedings of the Royal Society of London 353: 177-186.

Aris-Brosou, S. and Excoffier, L. 1996. The impact of population expansion and mutation rate heterogeneity on DNA sequence polymorphism. Molecular Biology and Evolution 13: 494-504.

Avise, J.C. and Walker, D. 1998. Pleistocene phylogeographic effects on avian populations and the speciation process. Proceedings of the Royal Society of London B265: 457-463.

Baker A.J. and Marshall, H.D. 1997. Mitochondrial control region sequences as tools for understanding evolution. *In* Avian molecular evolution and systematics. D.P. Mindell ed. Academic Press, San Diego. Pp 51-82.

Ballard, J.W. and Whitlock, M.C. 2004. The incomplete natural history of mitochondria. Molecular Ecology 13: 729-744.

Bechet, A., Martin, J., Meister, P. and Rabouam, C. 2000. A second breeding site for Ross's Gull (*Rhodostethia rosea*) in Nunavut, Canada. Arctic 53: 234-236.

Blomqvist, S. and Elander, M. 1981. Sabine's Gull (*Xema sabini*), Ross's Gull (*Rhodostethia rosea*) and Ivory Gull (*Pagophila eburnea*) Gulls in the Arctic: A Review. Arctic 34: 122-132.

Bouzat, J.L., Lewin, H.A. and Paige, K.N. 1998. The ghost of genetic diversity past: Historical DNA analysis of the Greater Prairie Chicken. American Naturalist 152: 1-6.

Buturlin, S.A. 1906. The breeding-grounds of the Rosy Gull. Ibis 8: 131-139, 333-337, 661-666.

Chartier, B. and Cooke, F. 1980. Ross's gulls (*Rhodostethia rosea*) nesting at Churchill, Manitoba, Canada. American Birds 34: 839-841.

Crochet, P-A. and Desmarais, E. 2000. Slow rate of evolution in the mitochondrial control region of Gulls (Aves: Laridae). Molecular Biology and Evolution 17: 1797-1806.

Conservation of Arctic Flora and Fauna (CAFF). 2004. Arctic Flora and Fauna: Status and Conservation. 250pp.

Cooper, A. 1994. DNA from museum specimens. In: Herrmann B., Herrmann, S. (eds) Ancient DNA: recovery and analysis of genetic material from paleontological, archeological, museum, medical and forensic specimens. Springer, New York.

Degtyarev, A.G., Labutin, Y.V. And Blohin Y.Y. 1987. Ross's gull *Rhodostethia rosea*: Notes on its migration and on peculiarities in its reproductive cycle near the limit of its distribution. Zoological Journal 66: 1873-1885.

Densley, M. 1999. In Search of Ross's Gull. Peregrine Books. 268pp.

Divoky, G., Sanger, G., Hatch, S. and Haney, J.C. 1988. Fall migration of Ross's gull *Rhodostethia rosea* in Alaskan Chukchi and Beaufort Seas. OCS Study, MMS 88-0023. Anchorage: Alaska Fish and Wildlife Research Centre. 120p.

Excoffier, L., Laval, G. and Schneider, S. 2005 Arlequin ver. 3.0: An integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online 1:47-50.

Excoffier, L., Smouse, P.E. and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479-491.

Freeland, J.R., Anderson, S., Allen, D. and Looney, D. 2007. Museum samples provide novel insights into the taxonomy and genetic diversity of Irish Red Grouse. Conservation Genetics 8: 695-703.

Fu, Y.X. 1997. Statistical neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147: 915-925.

Godoy, J.A, Negro, J., Hiraldo, F. and Donazar, J. 2004. Phylogeography, genetic structure and diversity in the endangered Bearded Vulture (*Gypaetus barbatus*, L.) as revealed by mitochondrial DNA. Molecular Ecology 13: 371-390.

Hewitt, G.M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biological Journal of the Linnaean Society 58: 247-276.

Hjort, C., Gudmundsson, G. and Elander, M. 1997. Ross's Gulls in the central Arctic Ocean. Arctic 50: 289-292.

Liebers, D. and Helbig, A.J. 2002. Phylogeography and colonization history of Lesser Black-backed Gulls (*Larus fuscus*) as revealed by mtDNA sequences. Journal of Evolutionary Biology 15: 1021-1033.

Liebers, D., Helbig, A.J. and De Kniff, P. 2001. Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* group (Aves: Charadriiformes). Molecular Ecology 10: 2447-2462.

Kampp, K. and Kristensen, K.M. 1980. Ross's Gull *Rhodostethia rosea* breeding in Disko Bay, West Greenland, 1979. Dansk orn. Foren. Tidsskr. 74: 65-74.

MacDonald, S.D. 1978. First breeding of Ross's gull in Canada. Proceedings of the Colonial Waterbird Group 1978: 16.

Mallory, M.L., Gilchrist, H.G. and Mallory, C.L. 2006. Ross's Gull (*Rhodostethia rosea*) Breeding in the Penny Strait, Nunavut, Canada. Arctic 59: 319-321.

Mallory, M.L., Akearok, J. and Fontaine, A.J. 2001. Community knowledge on the distribution and abundance of species at risk in southern Baffin Island, Nunavut, Canada. Canadian Wildlife Service Technical Report No. 363. 68p.

Martin, P.R. and McKay, J.K. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. Evolution 58: 938-945.

Maruyama, T. and Fuerst, P.A. 1985. Population bottlenecks and non-equilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. Genetics 111: 675-689.

Meltofte, H., Edelstam, C., Granstrom, G., Hammer, H. and Hjort, C. 1981. Ross's gulls in the Arctic pack-ice. British Birds 74: 316-320.

Moum, T. and Arnason, E. 2001. Genetic diversity and population history of two related seabird species based on mitochondrial DNA control region sequences. Molecular Ecology 10: 2463-2478.

Nettleship, D.N. and Evans, P.G.H. 1985. Distribution and status of the Atlantic Alcidae. In: The Atlantic Alcidae (eds Nettleship, D.N. and Birkhead, T.R.), pp. 53-154. Academic Press, New York.

Patirana, A., Hatch, S.A. and Friesen, V.L. 2002. Population differentiation in the Red-legged Kittiwake (*Rissa brevirostris*) as revealed by mitochondrial DNA. Conservation Genetics 3: 335-340.

Patirana, A. 2000. Global population genetics and evolutionary history of Black-legged Kittiwakes (*Rissa tridactyla*) as revealed by mitochondrial and nuclear DNA. Unpublished M.Sc. thesis. Queen's University.

Pearce, J.M., Talbot, S.L., Petersen, M.R. and Rearick, J.R. 2005. Limited genetic differentiation among breeding, molting, and wintering groups of the threatened Steller's Eider: the role of historic and contemporary factors. Conservation Genetics 6: 743-757.

Pons, J-M., Hassanin, A. and Crochet, P-A. 2005. Phylogenetic relationships within the *Laridae* (Charadriiformes: *Aves*) inferred from mitochondrial markers. Molecular Phylogenetics and Evolution 37: 686-699.

Reed, D.H., Briscoe, D.A. and Frankham, R. 2002. Inbreeding and extinction: The effect of environmental stress and lineage. Conservation Genetics 3: 301-307.

Rozas, J. and Rozas, R. 1999. DNASP, Version 3: an integrated program for molecular population genetics and molecular evolution analysis. Bioinformatics 15: 174-175.

Ruokonen, M., Aarvak, T. and Madsen, J. 2005. Colonization history of the high-arctic Pink-footed Goose *Anser brachyrhynchus*. Molecular Ecology 14: 171-178.

Ruokonen, M., Kvist, L., Aarvak, T., Markkola, J., Morozov, V., Oien, I., Syroechkovsky, E., Tolvanen, P. and Lumme, J. 2004. Population genetic structure and conservation of the Lesser White-fronted Goose *Anser erythropus*. Conservation Genetics 5: 501-512.

Schneider, S. and Excoffier, L. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152: 1079-1089.

Sefc, K.M., Payne, R.B. and Sorenson, M.D. 2007. Single base errors in PCR products from avian museum specimens and their effect on estimates of historical genetic diversity. Conservation Genetics 8: 879-884.

Slack, K.E., Delsuc, F., Mclenachan, P., Arnason, U. and Penny, D. 2007. Resolving the root of the avian mitogenomic tree by breaking up long branches. Molecular Phylogenetics and Evolution 42: 1-13.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123: 585-595.

Wilson, A.C., Cann, R., Carr, S., George, M., Gyllensten, U., Helm-Bychowski, K., Higuchi, R., Palumbi, S., Prager, E., Sage, R. and Stoneking, M. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of the Linnean Society 26: 375-400.

Wright, S. 1951. The genetical structure of populations. Ann. Eugen. 15: 323-354

Zink, R.M., Rohwer, S., Andreev, A.V. and Dittmann, D.L. 1995. Trans-Beringia comparisons of mitochondrial DNA differentiation in birds. Condor 97: 639-649.

Zubakin, V.A. *et al.* 1990. *Rhodostethia rosea* Rosenmöve. In: Ilichev, V.D. and Zubakin, V.A., eds. Handbuch der Vögel der Sovejetunion 6. Wittenberg: A. Ziemsen Verlag.

Zubakin, V.A. and Avdanin, V.O. 1983. Peculiarities of colonial nesting in *Rhodostethia rosea*. Zoologicheskij Zhurnal 62: 1754-1756.

Table 3.3.1 Polymorphic sites in the Control Region of Ross's Gull

		Base Pair											
	Sample#	18	30	42	106	115	283	294	312	314	355	380	434
Canada	60081	C	A	C	G	C	C	**T**	A	G	C	C	C
	60082	C	A	C	G	C	C	**T**	A	G	C	C	C
	60083	C	A	C	G	C	C	C	A	G	C	C	C
	70031	C	A	C	G	C	C	**T**	A	G	C	C	C
	86167	C	A	C	G	C	C	C	A	G	C	C	C
	3791	C	A	C	G	C	C	**T**	A	G	C	C	C
	3792	C	A	C	G	C	C	**T**	A	G	C	C	C
	4260	C	A	C	G	C	C	C	A	G	C	C	C
Alaska	158717	C	A	C	G	C	C	**T**	A	G	C	C	C
	160702	**T**	**G**	C	G	C	C	C	A	G	C	C	**T**
	160703	C	A	C	G	C	C	C	A	G	C	C	C
	160709	C	A	**T**	G	**T**	**T**	C	A	**A**	C	C	C
	160710	C	A	C	G	C	C	C	A	G	**A**	**T**	C
	1589296	C	A	C	**A**	C	C	C	**G**	**A**	C	C	C

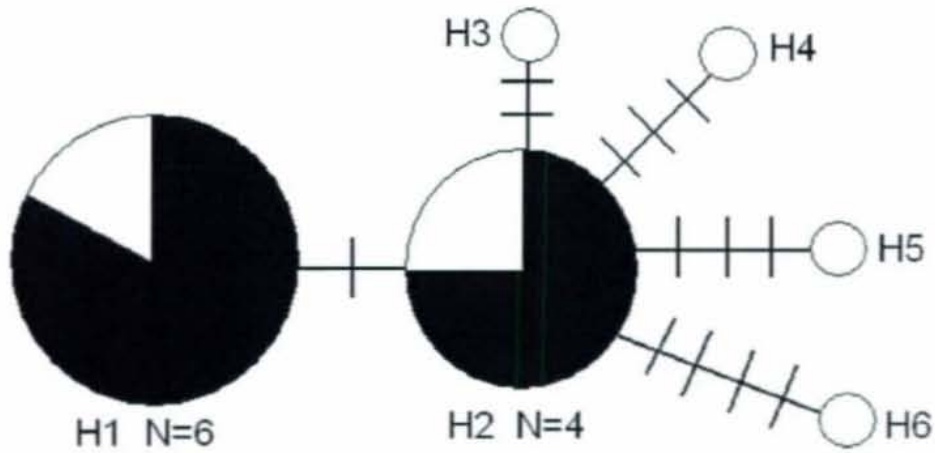

Figure 3.3.1 Ross's Gull Control Region Minimum Spanning Network

The minimum spanning network of Ross's Gull control region with Canadian birds in black and Alaskan birds in white.

Table 3.3.2 Ross's Gull Control Region genetic diversity by location

	Canada	Alaska	Total
Number of Individuals (N)	8	6	14
Haplotypes (H)	2	6	6
Haplotype Diversity (Hd)	0.535	1.00	0.769
Nucleotide Diversity (π)	0.00100	0.00820	0.00430
Average Number of Differences (k)	0.540	4.20	2.11

Table 3.3.3 Analysis of molecular variance (AMOVA) of Ross's Gull Control Region sequence data

Source of Variation	Degree of Freedom	Sum of Squares	Variance Component	Fraction of Variance	P-values
Among populations	1	2.05	0.149	0.126	0.0401
Within populations	12	12.4	1.03	0.873	-
Total	13	14.4	1.18	1.00	-

Table 3.3.4 Neutrality tests with Ross's Gull Control Region sequence data

	Canada	Alaska	Overall
Tajima's D	1.17	-1.22	-1.64
P-value	0.934 ns	0.112 ns	$0.05 \leq p \leq 0.10$ ns
Fu's F	0.866	-2.52	-2.29
P-value	0.578 ns	0.0370 *	$0.05 \leq p \leq 0.10$ ns

Table 3.4.1 Comparison of Ross's Gull genetic diversity values with relevant avian species

Species	Relevance	Nucleotide Diversity	Haplotype Diversity	Reference
Ross's Gull (*Rhodostethia rosea*)	-	0.00430	0.769	Chapter 3 of this thesis
Ivory Gull (*Pagophila eburnea*)	-	0.00207	0.451	Chapter 2 of this thesis
Red-legged Kittiwake (*Rissa brevirostris*)	Close relative	0.015	0.91	Patirana *et al.* 2002
Common Eider (*Somateria mollissima*)	Arctic species	0.0175	0.92	Tiedemann *et al.* 2004
Pink-footed Goose (*Anser brachyrhynchus*)	Arctic species	0.003	0.51	Ruokonen *et al.* 2005
Rock Ptarmigan (*Lagopus mutus*)	Arctic species	0.002	0.70	Holder *et al.* 2000
Three-toed Woodpecker (*Picoides tridactylus*)	Arctic species	0.001	0.63	Zink *et al.* 2002
Razorbill (*Alca torda*)	Arctic species	0.0126	0.92	Moum and Arnason 2001
Common Murre (*Uria aalge*)	Arctic species	0.005	0.72	Moum and Arnason 2001
Siberian Crane (*Grus leucogeranus*)	Endangered Arctic species	0.0060	0.9	Ponomarev *et al.* 2004
Whooping Crane (*Grus americana*)	Endangered species	0.0044	0.0045	Glenn *et al.* 1999
Crested Ibis (*Nipponia nippon*)	Endangered species	0.00069	0.386	Zhang *et al.* 2004
Heath Hen (*Tympanuchus cupido cupido*)	Extinct species	0.009	0.363	Johnson and Dunn 2006
Andean Condor (*Vultur gryphus*)	Scavenging species	0.0020	0.59	Hendrickson *et al.* 2003

Chapter 4- General Discussion, Future Directions and Recommendations

4.1 General Discussion

Molecular techniques, such as DNA sequencing, allow the quantification of genetic variation and partitioning of genetic variance among populations. These data in turn provide important information about population structure, population history and future research needs for species and populations of conservation concern. Acquisition of this information is especially important for endangered species where large gaps exist in the essential information needed to properly design a conservation strategy. Both Ivory Gull (*Pagophila eburnea*) and Ross's Gull (*Rhodostethia rosea*) are species of urgent conservation concern, in Canada, and require extensive research before a suitable management plan can be devised.

Conservation Genetics of Ivory Gulls and Ross's Gulls

This study shows that Ivory Gulls breeding in Canada, Greenland and Norway are not genetically differentiable, and could therefore be considered a single management unit. As well, birds wintering in the Labrador Sea are not differentiated from these three breeding sites. In contrast, the non-breeding birds in Alaska were weakly differentiated from the other populations. Ivory Gulls had a low level of genetic diversity and neutrality tests had negative values, which indicates that Ivory Gulls are expanding from a historical

population bottleneck. The estimated long-term effective population size was similar to other arctic avian species. Hopefully, the information provided by this study (i.e. that Ivory Gulls are a panmictic population) will give conservation managers more options to increase population size and reduce the possibility of damage due to a catastrophic pollution event. For example, translocation of Ivory Gulls between the breeding colonies of Canada and Greenland might become a useful tool to help increase the population size in Canada. Most important, my results provide further evidence for the small population size and fragile status of the Ivory Gull.

Mitochondrial DNA sequence analysis of Ross's Gull Control Region suggests that the Alaskan/Russian population and the Canadian population are weakly differentiated. As expected, the smaller Canadian population had much lower haplotype and nucleotide diversity than the larger Alaskan/Russian population. The cause of the genetic differentiation is not known and more research needs to be done before determining whether or not the Canadian population is a recent founder population or has existed at low numbers for a long time.

Avian Conservation Genetics

Due to the high dispersal abilities of birds, it can be difficult to resolve their population structure, however multiple studies have demonstrated significant mtDNA geographic structure in various avian families, including several gull species (Black-legged Kittiwakes (*Rissa tridactyla*) Patirana 2000; Red-legged Kittiwakes (*Rissa brevirostris*) Patirana, Hatch and Friesen 2002; Lesser Black-backed Gull (*Larus fuscus*) Liebers, Helbig and De Kniff 2001; and the Herring Gull complex (*Larus cachinnans-*

fuscus Liebers and Helbig 2002). This study provides more insight into population genetic structure of gulls, endangered and circumpolar arctic birds. Both Ivory Gulls and Ross's Gulls had relatively low levels of genetic variance seen between various populations, which differs from the strong level of population differentiation seen using mtDNA sequences of the other gull species analyzed. It would be interesting to compare the population differentiation seen in Sabine's Gull (*Xema sabini*), another high-arctic breeding gull, with that seen in Ivory Gulls and Ross's Gulls.

The phylogeographic pattern seen in Ivory Gulls also raises the possibility that wintering site fidelity may have an effect on genetic structure. Since Ivory Gulls do not breed in Alaska, the reason for my Alaskan sample's genetic differentiation from the other wintering site in the Labrador Sea, as well as the breeding colonies is not known. One possible explanation is that Ivory Gulls are wintering site philopatric and that it is at the wintering site where pair-bonds are formed. This idea has been previously suggested for waterfowl (Robertson and Cooke 1999) but when tested with King Eiders (*Somateria spectabilis*), a species with both Pacific and Atlantic wintering areas, it was not supported as no significant genetic variance was found (Pearce *et al.* 2004). More research needs to be done to determine whether or not Ivory Gulls are wintering site philopatric and whether it is at this time when pair-bonds are formed, as these data are not currently available.

Genetic diversity is an important aspect of avian conservation genetics, and it has been shown to be correlated with fitness components (Reed and Frankham 2003) such as egg infertility and hatching failure (Bensch *et al.* 1994; Jamieson and Ryan 2000), a population's likelihood of recovery from bottlenecks (Frankham 1998), and the ability to

respond to environmental change (Reed, Briscoe and Frankham 2002). The results of this study support the notion that endangered and/or Arctic species have lower levels of genetic diversity (Spielman, Brook and Frankham 2004; Martin and McKay 2004), but the effect of this on the fitness of the Ivory Gull and Canadian Ross's Gull and their ability to adapt to the dynamic Arctic environment has not been researched. Some avian species have been able to survive despite reduction to a single breeding pair, for example the Chatham Island Black Robin (*Petroica traversi*), although they did show a higher rate of hatching failure (Ardern and Lambert 1997).

Museum samples are becoming an important source of genetic material for conservation genetic studies, as they are easy to obtain and can offer information on temporal trends of genetic parameters (Payne and Sorenson 2002). This study was the first conservation-oriented genomic analysis that used only museum samples, which demonstrates that they alone can be utilized to sequence large sections of the genome and provide fundamental data. As expected, the control region included the largest number of parsimony-informative SNPs, but other regions did possess them, which makes taking a genomic approach more accurate in assessing the true population structure and genetic diversity of a species.

4.2 Future Directions

Conservation Genetics

More comprehensive sampling of Ivory Gulls and Ross's Gulls would provide a more accurate assessment of the conservation genetics of both species. Samples of both Ivory Gulls and Ross's Gulls from breeding colonies in Russia would provide the most

new information as they were not available for the previous analysis. Increasing the samples from each population, especially of Ross's Gulls, would also allow an even more accurate assessment of the conservation genetic parameters and the genetic structure of each species. Obtaining current samples should also be a priority, especially from Ivory Gulls, as that would allow evaluation of current genetic diversity values. The use of hypervariable nuclear loci such as microsatellites or introns would also be beneficial as they are passed both maternally and paternally and thus would allow detection of sex-specific dispersal. These markers would also provide independent verification of the population genetic structure calculated using mitochondrial DNA.

Conservation Biology Research

There are several hypotheses about the reasons for the decline in Ivory Gulls but little or no research has been done to test them, despite Ivory Gulls being classified as Endangered in Canada (COSEWIC 2006). Since declines have occurred in all habitat types and across the known Canadian breeding range, Gilchrist and Mallory (2005) suggest that the cause of the decline is something that the colonies all have in common such as factors occurring during migration (e.g. hunting) or on the wintering grounds.

Hunting is thought to be an important factor, as the high band recovery rates for Ivory Gulls are comparable to other harvested birds (Stenhouse, Robertson and Gilchrist 2004), but more data are needed to fully understand the impact of hunting on the Ivory Gull population. Band recoveries from hunting may also help provide more data about Ivory Gull movements and help determine which populations are being killed with higher frequency.

Another possibility is that ecological changes may have occurred on the wintering grounds (Gilchrist and Mallory 2005). The sea ice distribution and thickness in the Northwest Atlantic is changing (Vinnikov *et al.* 1999; Drinkwater 2004), and it is possible that this is negatively affecting Ivory Gulls. If this is true it may influence the other breeding colonies as well, since the Labrador Sea is the main wintering areas for Ivory Gulls. The birds that winter in Alaska also need further research, as these were the only differentiated population found in this study. Unlike many other Arctic-breeding seabirds, Ivory Gulls feed in association with sea-ice year-round (Haney and MacDonald 1995). Reproductive output has been seen to be smaller in years of less ice (Dalgety 1932) and they may be particularly sensitive to reduction in sea ice as a result of climate warming (Vinnikov *et al.* 1999). Ivory Gulls are considered to be an indicator species of the health of the marine environment by the Inuit and scientists (Mallory and Gilchrist 2005).

Ivory Gulls have some of the highest known values of contamination of many toxic chemicals, including PCBs, DDT (Fisk, Hobson and Norstrom 2001; Buckman *et al.* 2004) and methylmercury (Braune, Mallory and Gilchrist 2006). The potentially deleterious effect these chemicals are having on reproductive success and other parameters are not known. Gulls are considered to be highly vulnerable to oil pollution (Camphuysen 1998) and Ivory Gulls and Ross's Gulls are more pelagic than most, making them even more susceptible yet less likely to be recovered on land (COSEWIC 2006).

There is no data available to indicate whether the declines in Canada are also being seen in Ivory Gulls from other breeding areas. This will need to be an international

undertaking, with censuses done in Greenland, Svalbard and several areas in Russia, such as Severnaya Zemlya, Novaya Zemlya and Franz Josef Land. Banding programmes at the breeding colonies and wintering areas would help provide a more accurate description of movement between breeding colonies, wintering area usage and hunting mortality.

It is extremely important to quantify Ivory Gull demographic parameters as without this information, the potential for recovery of the species cannot be accurately gauged (Stenhouse 2004). Ivory Gull breeding biology research will provide information on essential parameters such as breeding success, extent of philopatry, age at first breeding and adult survival. Ivory Gulls have several known predators during the breeding season but data is needed on the rate of predation and the extent of variation between years (COSEWIC 2006).

A more accurate assessment of the number of Ross's Gulls breeding in Canada is needed. Since the only study done on Ross's Gull breeding biology was done by Buturlin in 1906, research needs to be done to determine critical demographic parameters in both Russia and Canada. The effects of predation, disturbance and adverse weather need to be resolved in order to accurately assess the potential recovery of this species. The wintering areas of Ross's Gulls breeding in both in Russia and in Canada needs to be more accurately established so that the populations can be properly protected, especially against potential oil pollution in Alaskan waters.

4.3 Recommendations

Ivory Gulls in North American breeding colonies are not genetically differentiated from those in western European breeding colonies, which indicates that Ivory Gulls could

be considered a single management unit. Canadian Ross's Gulls are genetically differentiated from Alaskan Ross's Gulls which suggests that they may be considered as a separate management unit. However, this latter conclusion is based on a small number of samples so further work should be done to confirm this.

The results of my study underline the urgent need for the Canadian federal government to implement regulatory policies that will protect Ivory Gulls and Ross's Gulls. This includes (but is not limited to): 1) absolute protection of Ivory Gulls and Ross's Gull from hunting in Canada and education programs for hunters and Inuit communities in general so that they understand the threat these species face; 2) absolute protection of Ivory Gull and Ross's Gull breeding colonies from human-caused disturbance such as industrial activities or tourism; 3) increased surveillance and enforcement of marine pollution laws (e.g. Bill C-15) to minimize the chance of Ivory Gulls being oiled at sea in their wintering areas; and 4) rapid implementation of controls of greenhouse gas emissions that are causing global warming and associated rapid climate change in the Arctic. Since the Ivory Gull and Ross's Gull breed internationally, every effort should be made to have these policies adopted with other relevant countries. Long term persistence of the Ivory Gull and Canadian Ross's Gull seems grim, but extinction seems likely unless the above measures are taken promptly.

References

Ardern, S.L. and Lambert, D.M. 1997. Is the Black Robin in genetic peril? Molecular Ecology 6: 21-28.

Bensch S., Hasselquist, D. and Von Schantz, T. 1994. Genetic similarity between parents predicts hatching failure: Non-incestuous breeding in the great reed warbler. Evolution 48: 317-326.

Braune, B.M., Mallory, M.L. and Gilchrist, H.G. 2006. Elevated mercury levels in a declining population of Ivory Gulls in the Canadian Arctic. Marine Pollution Bulletin 52: 969-987.

Buckman, A. H., Norstrom, R., Hobson, K., Karnovsky, N., Duffe, J. and Fisk, A. 2004. Organochlorine contaminants in seven species of Arctic seabirds from northern Baffin Bay. Environmental Pollution 128: 327-338.

Buturlin, S.A. 1906. The breeding-grounds of the Rosy Gull. Ibis 8: 131-139, 333-337, 661-666.

Camphuysen, K. 1998. Beached bird surveys indicate decline in chronic oil pollution in the North Sea. Marine Pollution Bulletin 36: 519-523.

COSEWIC. 2006. COSEWIC assessment and update status report on the Ivory Gull *Pagophila eburnea* in Canada. Committee on the Status of Endangered Wildlife in Canada. Ottawa. vi + 42p.

Dalgety, C.T. 1932. The Ivory Gull in Spitsbergen. British Birds 26:2-7.

Drinkwater, K.F. 2004. Atmospheric and sea-ice conditions in the Northwest Atlantic during the decade 1991-2000. Journal of Northwest Atlantic Fishery Science 34: 1-11.

Fisk, A., Hobson, K. and Norstrom, R. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. Environmental Science & Technology 35: 732-738.

Frankham, R. 1998. Inbreeding and extinction: Island populations. Conservation Biology 12: 665-675

Gilchrist, H.G. and Mallory, M. 2005. Declines in abundance and distribution of the Ivory Gull (*Pagophila eburnea*) in Arctic Canada. Biological Conservation 121: 303-309.

Haney, J.C. and MacDonald, S.D. 1995. Ivory Gull (*Pagophila eburnea*). In The Birds of North America, No. 175 (Poole, A. and Gill, F., eds). The Birds of North America, Inc., Philadelphia.

Jamieson, I.G. and Ryan, C.J. 2000. Increased egg infertility associated with translocating inbred Takahe (*Porphyrio hochstetteri*) to island refuges in New Zealand. Biological Conservation 94: 107-114.

Liebers, D. and Helbig, A.J. 2002. Phylogeography and colonization history of Lesser Black-backed Gulls (*Larus fuscus*) as revealed by mtDNA sequences. Journal of Evolutionary Biology 15: 1021-1033.

Liebers, D., Helbig, A.J. and De Kniff, P. 2001. Genetic differentiation and phylogeography of gulls in the *Larus cachinnans-fuscus* group (Aves: Charadriiformes). Molecular Ecology 10: 2447-2462.

Mallory, M. and Gilchrist, G. 2005. Population trends and habitat requirements of Ivory Gulls breeding in Nunavut. Ivory Gull 2005 Report. Canadian Wildlife Service. 11pp.

Martin, P.R. and McKay, J.K. 2004. Latitudinal variation in genetic divergence of populations and the potential for future speciation. Evolution 58: 938-945.

Mehlum, F. 1990. Seabird distribution in the northern Barents Sea marginal ice-zone during late summer. Polar Research 8: 61-65.

Patirana, A., Hatch, S.A. and Friesen, V.L. 2002. Population differentiation in the Red-legged Kittiwake (*Rissa brevirostris*) as revealed by mitochondrial DNA. Conservation Genetics 3: 335-340.

Patirana, A. 2000. Global population genetics and evolutionary history of Black-legged Kittiwakes (*Rissa tridactyla*) as revealed by mitochondrial and nuclear DNA. Unpublished M.Sc. thesis. Queen's University.

Payne, R.B. and Sorenson, M.D. 2002. Museum collections as sources of genetic data. In: R. van den Elzen (ed) Bird Collections in Europe: promotion of science in ornithology. Alexander Koenig Research Institute and Museum of Zoology Proceedings, Vol. 2, Bonn, Germany.

Pearce, J.M., Talbot, S., Pierson, B., Petersen, M., Scribner, K., Dickson, D.L. and Mosbech, A. 2004. Lack of spatial genetic structure among nesting and wintering King Eiders. Condor 106: 229-240.

Reed, D.H. and Frankham, F. 2003. Correlation between Fitness and Genetic Diversity. Conservation Biology 17: 230-237.

Reed, D.H., Briscoe, D.A. and Frankham, F. 2002. Inbreeding and extinction: The effect of environmental stress and lineage. Conservation Genetics 3: 301-307.

Robertson, G.J. and Cooke, F. 1999. Winter philopatry in migratory waterfowl. Auk 116: 20-34.

Spielman, D., Brook, B.W. and Frankham, R. 2004. Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences 101: 15261-15264.

Stenhouse, I.J., Robertson, G.J. and Gilchrist, H.G. 2004. Recoveries and survival rates of Ivory Gulls (*Pagophila eburnea*) banded in Nunavut, Canada, 1971-1999. Waterbirds 27: 486-492.

Stenhouse, I.J. 2004. Canadian management plan for the Ivory Gull (*Pagophila eburnea*). Canadian Wildlife Service, St. John's, NL.

Stenhouse, I.J. 2003. Ivory Gull Conservation Strategy and Action Plan. Canadian Ivory Gull Working Group.

Vinnikov, K.Y., Robock, A., Stouffer, R., Walsh, J., Parkinson, C., Cavalieri, D., Mitchell, J., Garrett, D. and Zakharov, V. 1999. Global warming and Northern Hemisphere sea ice extent. Science 286: 1934-1937.

Appendix 1: Ivory Gull sample information including location, date, museum and markers sequenced

Canada Breeding samples

Museum Sample	Region	Location	Year	Day	CR	12S rRNA	4-1	4L
CMN06704	Nunavut	N63.97E61.82	1904	Sep22	No	Yes	Yes	Yes
CMN23444	Nunavut	Dundas Harbour	1929	Aug6	Yes	Yes	No	Yes
CMN57268	Nunavut	N74.68E94.83	1969	July4	Yes	Yes	Yes	Yes
CMN69123	Nunavut	N79.10E75.87	1979	Jun 18	Yes	Yes	Yes	Yes
CMN69124	Nunavut	N79.10E75.87	1979	Jun 18	Yes	Yes	Yes	Yes
CMN69193	Nunavut	Pond Inlet	1978	Jun14	Yes	Yes	Yes	Yes
CMN69194	Nunavut	Pond Inlet	1978	July	Yes	Yes	Yes	Yes
CMN69195	Nunavut	Pond Inlet	1978	July	Yes	Yes	Yes	Yes
CMN71689	Nunavut	Seymour Island	1975	Aug17	Yes	Yes	Yes	Yes
CMN71690	Nunavut	Seymour Island	1975	Jul30	Yes	Yes	Yes	Yes
CMN83462	Nunavut	N/A	N/A	N/A	Yes	Yes	Yes	Yes
CMN83464	Nunavut	Seymour Island	1976	Aug8	Yes	Yes	Yes	Yes
CMN83465	Nunavut	N/A	N/A	N/A	Yes	Yes	No	Yes
CMN83478	Nunavut	N/A	N/A	N/A	Yes	Yes	No	Yes
CMN83479	Nunavut	N/A	N/A	N/A	Yes	Yes	No	No
CMN83480	Nunavut	N/A	N/A	N/A	Yes	Yes	No	No
CMN83481	Nunavut	N/A	N/A	N/A	Yes	Yes	No	No
CMN83483	Nunavut	Seymour Island	1977	July2	Yes	Yes	Yes	Yes
CMN83633	Nunavut	N/A	N/A	N/A	Yes	Yes	Yes	Yes
CMN84135	Nunavut	N/A	N/A	N/A	Yes	Yes	Yes	Yes
CMN84138	Nunavut	Grise Fjord	1983	July9	Yes	Yes	Yes	Yes
CMN84139	Nunavut	N/A	N/A	N/A	Yes	Yes	Yes	Yes
ROM79400	Nunavut	Baffin Island	1951	Jun29	Yes	Yes	Yes	Yes
CM129250	NWT	Inuvik District	1941	Sep	No	No	Yes	Yes
CM161539	Nunavut	Baffin District	1937	Aug17	Yes	No	Yes	No
ANSP118867	Nunavut	Ellesmere Island	1934	Sep8	Yes	Yes	Yes	Yes
ANSP118869	Nunavut	Ellesmere Island	1934	Sep8	Yes	No	No	No

ANSP118872	Nunavut	Ellesmere Island	1934	Sep8	Yes	Yes	Yes	No

Canada Non-Breeding samples

Museum Sample	Region	Location	Year	Day	CR	12S rRNA	4-1	4L
IVGU1	NL	N/A	2000s	N/A	Yes	Yes	Yes	Yes
IVGU2	NL	N/A	2000s	N/A	Yes	Yes	Yes	Yes
FA	NL	Fogo Island	2000s	winter	Yes	Yes	Yes	Yes
FJ	NL	Fogo Island	2000s	winter	Yes	Yes	Yes	Yes
B/I	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
PL6	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-IL1	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-IL2	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-IL3	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-IL4	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-IL5	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL1	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	No	No
CMN-AL2	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL3	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL4	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL5	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL6	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL7	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL8	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL9	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL10	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN-AL11	NL	L'Anse Aux Meadows	1980s	winter	Yes	Yes	Yes	Yes
CMN29210	Quebec	Natashquan	1939	Dec19	Yes	Yes	Yes	Yes
CMN29217	Quebec	N/A	N/A	N/A	Yes	No	No	No
CMN65716	NWT	N70.17E116.50	1976	Nov	Yes	Yes	Yes	Yes
ROM75016	Ontario	Kenora District	1956	Jan	Yes	Yes	Yes	Yes
ANSP146497	U.S.	New Jersey	1940	Feb	Yes	No	No	No

Norway Breeding samples

Museum Samples	Region	Location	Year	Day	CR	12S rRNA	4-1	4L
OSLO7401	Norway	Biskayerhuken	1949	Jul14	Yes	Yes	Yes	Yes
OSLO7402	Norway	Biskayerhuken	1949	Jul12	Yes	No	No	Yes
OSLO7403	Norway	Bjornehavn	1949	Jul3	Yes	No	Yes	No
OSLO7537	Norway	Mosselbay	1954	Jul28	Yes	No	Yes	Yes
OSLO11145	Norway	Svalbard	1982	Aug20	Yes	Yes	Yes	Yes
OSLO11148	Norway	Svalbard	1982	Aug20	Yes	No	Yes	Yes
OSLO11149	Norway	Svalbard	1982	Aug20	Yes	Yes	Yes	Yes
OSLO11150	Norway	Svalbard	1982	Aug20	Yes	Yes	Yes	No
OSLO11166	Norway	Svalbard	1982	Aug20	Yes	Yes	Yes	Yes
OSLO11410	Barents Sea	N76.45E29.0	1986	May25	Yes	Yes	Yes	Yes
ZMUC28.912	Norway	Siktefjeld	1949	Jul25	Yes	No	No	No
ZMUC28.913	Norway	Biskayernuken	1949	Jul15	Yes	Yes	Yes	Yes
ZMUB2115	Norway	Finnmark	N/A	N/A	Yes	Yes	No	No
ZMUB10375	Norway	Svea	1947	Jul25	Yes	Yes	Yes	Yes
ZMUB10376	Norway	Svea	1947	Jul25	Yes	Yes	Yes	Yes
ZMUB10379	Norway	Svea	1947	Jul25	Yes	Yes	Yes	Yes
ZMUB11629	Norway	Kong Karls Land	1960	Aug7	Yes	Yes	Yes	Yes

Greenland Breeding samples

Museum Sample	Region	Location	Year	Day	CR	12S rRNA	4-1	4L
ZMUC14.147	Greenland	Isenv. Kap Stephensen	1932	Jul22	Yes	Yes	Yes	Yes
ZMUC14.148	Greenland	Isenv. Kap Stephensen	1932	Jul22	Yes	Yes	Yes	Yes
ZMUC14.149	Greenland	Isenv. Kap Stephensen	1932	Jul23	Yes	Yes	No	Yes
ZMUC14.150	Greenland	Isenv. Kap Stephensen	1932	Jul23	Yes	Yes	Yes	Yes
ZMUC14.151	Greenland	Isenv. Kap Stephensen	1932	Aug15	Yes	Yes	Yes	Yes
ZMUC14.190	Greenland	Scoresby Lund	1933	Aug25	Yes	Yes	Yes	Yes
ZMUC57.689	Greenland	Kane Basin	1941	Jun5	Yes	Yes	Yes	Yes
ZMUC57.690	Greenland	Kane Basin	1941	Jun5	Yes	Yes	Yes	Yes
ZMUC57.691	Greenland	Kane Basin	1941	Jun5	Yes	Yes	Yes	Yes

ZMUC57.692	Greenland	Kane Basin	1941	Jun5	Yes	No	Yes	Yes
ANSP118864	Greenland	Melville Bay (W)	1934	Jul31	Yes	Yes	No	Yes
CM161522	Greenland	N/A	1937	Jul24	Yes	No	Yes	Yes
CM161523	Greenland	N/A	1937	Aug3	Yes	Yes	Yes	Yes
CM161524	Greenland	N/A	1940	Aug8	Yes	Yes	Yes	Yes
MVZ101400	Greenland	Melville Bay	1925	Jul30	Yes	Yes	No	No

Greenland Non-Breeding samples

Museum Samples	Region	Location	Year	Day	CR	12S rRNA	4-1	4L
ZMUC14.145	Greenland	Isenv. Kap Stephensen	1934	Jan	Yes	Yes	Yes	Yes
ZMUC55.659	Greenland	Holsteinborg	1935	May21	Yes	Yes	No	Yes
ZMUC57.693	Greenland	Smith's Sound	1940	May	Yes	Yes	Yes	Yes
ZMUC57.694	Greenland	Smith's Sound	1940	May	Yes	Yes	Yes	Yes
ZMUC57.695	Greenland	Smith's Sound	1940	May	Yes	Yes	Yes	Yes
ZMUC64.171	Greenland	Angmaqssalik	1976	N/A	Yes	Yes	Yes	Yes
ZMUC64.215I	Greenland	Sarqaq, Disko	1948	Dec	Yes	Yes	Yes	Yes
ZMUC64.217	Greenland	Sarqaq, Disko	1948	Dec	Yes	Yes	Yes	Yes
ZMUC65.822	Greenland	Godthaab	1964	Jan	Yes	Yes	Yes	Yes
ZMUB3904	Greenland	Godhavn	1907	Nov5	Yes	Yes	Yes	Yes
MVZ101401	Greenland	Egedesminde	1925	Sep18	Yes	Yes	Yes	Yes

Alaska Non-Breeding samples

Museum Samples	Region	Location	Year	Day	CR	12S rRNA	4-1	4L
CRCM76-474	Alaska	N/A	1976	Apr17	Yes	Yes	Yes	Yes
FM158416	Alaska	Barrow	1928	Sep11	Yes	No	No	Yes
FM158417	Alaska	Barrow	1930	May19	Yes	No	No	Yes
FM158418	Alaska	Barrow	1929	Oct25	Yes	Yes	No	Yes
FM158420	Alaska	Barrow	1930	May19	Yes	Yes	No	Yes
FM158421	Alaska	Barrow	1929	Oct12	Yes	No	No	No
FM158423	Alaska	Barrow	1929	Oct7	Yes	No	No	No

FM158424	Alaska	Barrow	1931	Sep26	Yes	Yes	No	Yes
FM158425	Alaska	Barrow	1930	May19	Yes	No	No	Yes
FM158431	Alaska	Barrow	1927	Oct11	Yes	Yes	No	No
FM160631	Alaska	Barrow	1931	Oct6	Yes	No	No	No
FM160632	Alaska	Barrow	1929	Oct11	Yes	No	No	No
FM160633	Alaska	Barrow	1929	Oct7	Yes	No	No	Yes
FM160634	Alaska	Barrow	1930	May23	Yes	Yes	Yes	Yes
FM160635	Alaska	Barrow	1927	Oct5	Yes	Yes	Yes	Yes
FM160636	Alaska	Barrow	1927	Oct11	Yes	Yes	No	Yes
FM160637	Alaska	Barrow	1927	Oct8	Yes	No	No	Yes
FM160638	Alaska	Barrow	1927	Oct11	Yes	No	No	Yes
FM160639	Alaska	Barrow	1927	Oct4	Yes	Yes	Yes	Yes
FM160640	Alaska	Barrow	1929	Oct4	Yes	No	No	Yes
FM160641	Alaska	Barrow	1927	Oct11	Yes	Yes	Yes	Yes
USNM253120	Alaska	St. George Island	1916	Feb18	Yes	No	No	No
USNM255117	Alaska	St. George Island	1917	Mar27	No	Yes	No	No
USNM469300	Alaska	Old Crow	1958	Jan26	Yes	Yes	No	No
USNM479604	Alaska	St. Paul Island	1962	Jan24	Yes	Yes	Yes	Yes
USNM479605	Alaska	St. Paul Island	1962	Jan25	Yes	Yes	Yes	Yes
USNM479606	Alaska	St. Paul Island	1962	Jan28	Yes	Yes	No	Yes
MVZ45096	Alaska	Wainwright	1924	May23	Yes	No	No	No
MVZ82095	Alaska	Barrow	1930	May29	Yes	No	No	No
ROM81716	Alaska	Barrow	1931	Sep25	Yes	No	No	No
UBC5647	Alaska	Barrow	1931	Sep25	Yes	No	No	No

Other samples

Museum Sample	**Region**	**Location**	**Year**	**Day**	**CR**	**12S rRNA**	**4-1**	**4L**

UWBM72711	Arctic Ocean	N/A	1993	Sep7	Yes	Yes	Yes	Yes

NL= Newfoundland and Labrador
NWT= Northwest Territories

Museum Abbreviations:
CMN=Canadian Museum of Nature
ROM=Royal Ontario Museum
CM=Carnegie Museum of Natural History
ANSP=Academy of National Sciences
OSLO=Natural History Museum, University of Oslo
ZMUC=Zoological Museum, University of Copenhagen
ZMUB= Museum of Zoology, University of Bergen
MVZ=Museum of Vertebrate Zoology, University of California
CRCM=Charles R. Connor Museum, Washington State University
FM=Field Museum of Natural History
USNM=Smithsonian Institution, National Museum of Natural History
UBC=Cowan Vertebrate Museum, University of British Columbia
UWBM=Burke Museum of Natural History, University of Washington

Appendix 2: Ross's Gull sample information including location, date, and museum

Canadian samples

Museum Sample	Region	Location	Year	Date
CMN60081	Nunavut	Seymour Island	1974	July 24
CMN60082	Nunavut	Seymour Island	1974	July 25
CMN60083	Nunavut	Seymour Island	1974	July 25
CMN70031	Newfoundland and Labrador	Fogo Island	1976	Dec. 18
CMN86167	Nunavut	Baffin Island	1985	June 14
MM3791	Manitoba	Churchill	1982	July 16
MM3792	Manitoba	Churchill	1982	July 17
MM4260	Manitoba	Churchill	1983	July 5

Alaskan samples

Museum Sample	Region	Location	Year	Date
FM158717	Alaska	Point Barrow	1931	Sept. 17
FM160702	Alaska	Point Barrow	1928	Oct. 2
FM160703	Alaska	Point Barrow	1928	Sept. 28
FM160709	Alaska	Point Barrow	1928	Oct. 2
FM160710	Alaska	Point Barrow	1929	Oct. 7
MVZ158296	Alaska	Singoalik River	1961	July 29

CMN= Canadian Museum of Nature
MM= Manitoba Museum
FM= Field Museum of Natural History
MVZ= Museum of Vertebrate Zoology, University of California

Control

1. FP♂ ×FP♀MP(male producing)
   OP♂ ×FP♀MP(male producing)
2. FP♂ ×FP♀NMP(none male producing)
   OP♂ ×FP♀NMP(none male producing)

Experiment

1. FP♂, OP♂ } ×FP♀MP(male producing)
2. FP♂, OP♂ } ×FP♀NMP(none male producing)

Control

1. FP♂ ×FP♀MP(male producing)
2. FP♂ ×FP♀NMP(none male producing)

Experiment

1. OP♂ ×FP♀MP(male producing)
2. OP♂ ×FP♀NMP(none male producing)